“ HER 2 neu EXPRESSION IN COLORECTAL ADENOCARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGIC VARIABLES”

Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2016

DECLARATION

I hereby declare that the dissertation entitled "**HER 2 neu expression in colorectal adenocarcinoma and its correlation with clinicopathologic variables** " is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from July 2014 to July 2015 under the guidance and supervision of **Dr. V.PRABA, M.D**., Associate Professor , Department of Pathology, Coimbatore Medical College.

This dissertation is submitted to The Tamilnadu Dr.MGR Medical University, Chennai towards the partial fulfilment of the requirement for the award of M.D., Degree (Branch III) in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

Place: Coimbatore

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CERTIFICATE

This is to certify that the dissertation entitled "HER 2 neu expression in colorectal adenocarcinoma and its correlation with clinicopathologic variables" is a record of bonafide work done by **Dr.N.VANI** in the Department of Pathology, Coimbatore Medical College, Coimbatore under the guidance and supervision of **Dr.V.PRABA, M.D**., Associate Professor, Department of Pathology, Coimbatore Medical College and submitted in partial fulfilment of the requirements for the award of M.D. Degree (Branch III) in Pathology by The Tamilnadu Dr. MGR Medical University, Chennai.

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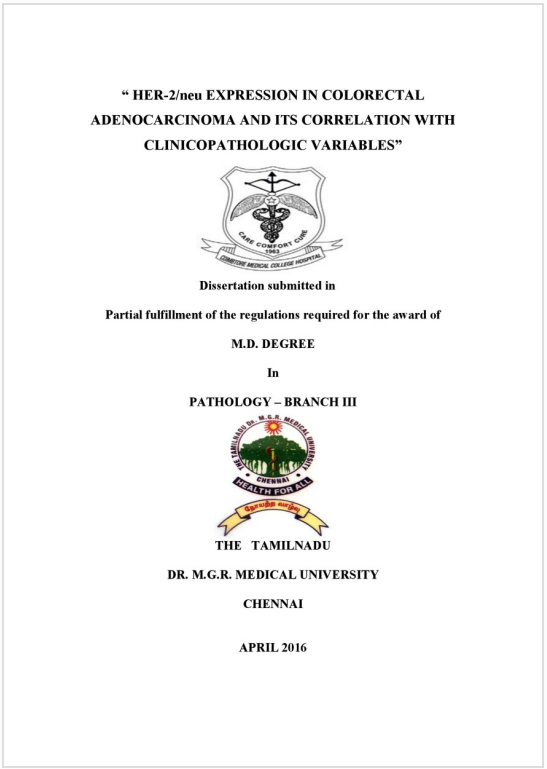

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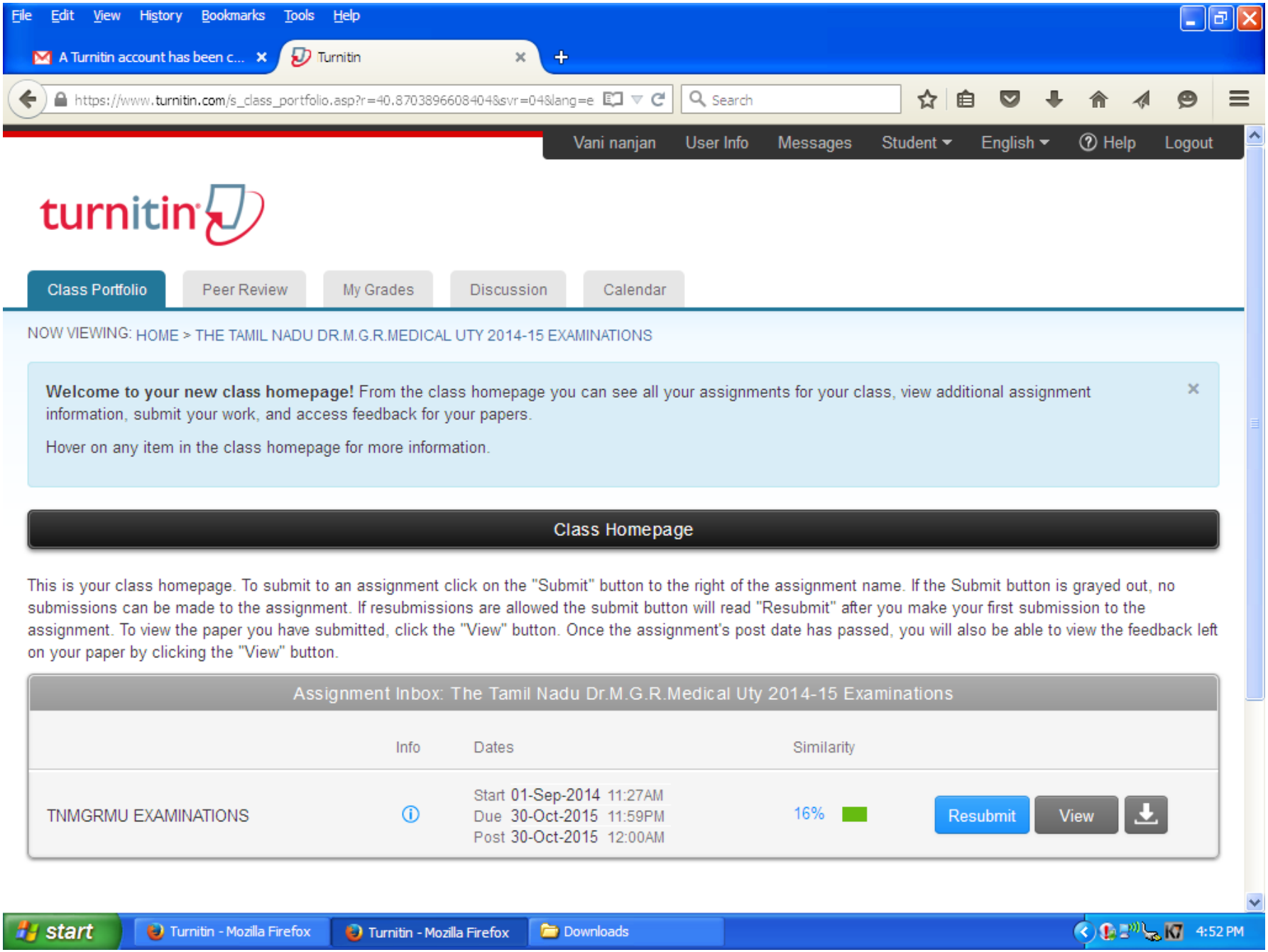
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INTRODUCTION

INTRODUCTION

Colorectal cancer is one among the most common malignancies throughout the world. Worldwide incidence rate of colon cancer is increasing to 2% annually. Accounts for 9% of all cancer incidence.

Etiology appears to be multifactorial which includes genetics, environmental factors and dietary factors. Peak incidence is in the seventh decade and a male predominance is seen. Sigmoid colon and rectum are the most commonly affected sites.

Inherited syndromes associated with increased risk of colorectal cancer include Lynch syndrome (formerly known as hereditary non-polyposis colorectal cancer), Familial adenomatosis polyposis, Peutz-Jeghers syndrome and PTEN Hamartoma syndrome (Cowden syndrome). Chronic inflammatory bowel diseases (ulcerative colitis and crohns disease) are also associated with an increased risk for colon cancer.

Colorectal carcinomas may be fungating, intraluminal or ulcerating masses. Adenocarcinomas account for more than 90% of colorectal carcinomas. Most common histological pattern is classic adenocarcinoma.

Other subtypes are :

- Mucinous adenocarcinoma
- Signet ring cell carcinoma
- Medullary carcinoma
- Serrated carcinoma
- Adenosquamous carcinoma
- Small cell carcinomas or neuroendocrine carcinomas

The grading system for colorectal adenocarcinomas is based on the extent of well-formed glands either well differentiated (>95%), moderately differentiated (50-95%) or poorly differentiated (<50%).

Histomorphological assessment on hematoxylin-eosin (H&E) stained sections remains the most important diagnostic tool.

Immunohistochemistry can be used for the diagnosis and prognosis of colorectal adenocarcinomas. Various immunological markers for colon carcinoma are MUCI and MUC3, P53, CK20, CEA, HER-2/neu, CD X2, tumour associated glycoprotein, cathepsin, HCG and PLAP.

Human epidermal growth factor receptor (HER-2/neu) over expression correlates with mitogenesis , malignant transformation , invasion and metastasis and correlates with poor prognosis . HER-2/ neu is located on chromosome 17q21 and it encodes a 185 KD transmembrane protein . HER-2/neu activity initiates signal cascades including MAPK (Mitogen activated protein kinase) and p13k (AKT 3 kinase) pathways that are essential for cell proliferation and differentiation.

The purpose of this study is to analyze the histomorphological patterns and grades of colorectal adenocarcinoma and to study immunohistochemical expression of HER-2/ neu and its correlation with clinico pathologic variables and its role in targeted therapy.

AIM AND OBJECTIVES

AIM AND OBJECTIVES

Aim is to study the correlation between HER-2/neu expression in colorectal adenocarcinoma.

OBJECTIVES

- To study HER-2/neu expression in colorectal adenocarcinomas.
- To study the correlation of HER-2 /neu expression with age, sex, site and type of tumour.
- To study the correlation of HER-2/neu expression with histological grade of carcinoma.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Colorectal carcinoma is one of the most deadly cancer with increased morbidity and mortality in the word.[1] Incidence of colorectal carcinoma is 9% and this being the third most common cancer world wide.[2] Classic adenocarcinomas accounts for 90% of colorectal carcinomas and it is the most common type. Mucinous adenocarcinoma, signet ring cell carcinoma, medullary carcinoma, serrated carcinoma, adenosquamous carcinoma and small cell carcinomas account for the remainder cases. In a study by Manmeet Kaur Gill et al (2011) showed that conventional adenocarcinoma consitituted about 77.5% followed by mucinous adenocarcinoma 17.5% and signet ring cell carcinoma 2.5%.[3]

HISTORICAL HIGHLIGHTS:

Colorectal carcinomas were not recognized as a distinct disease until 1960. Incidence rates have doubled since mid 1970's.[4] Incidence rate declined from mid 1980's to mid 1990's followed by a short period of stabilization . From 1998 to 2005 , incidence rate again declined – an average of 2.8% per year for men and 2.2% per year among woman. Mortality for CRC has

declined over the last 20 years. Between 1985 and 2002 the decline was 1.8% per year.

EMBRYOLOGY :

The gastrointestinal tract initially arises during the process of gastrulation from the endoderm of the trilaminar embryo (week 3) and extends from the buccopharyngeal membrane to the cloacal membrane . Three distinct regions – forgut, midgut and hindgut are formed during the 4^{th} week . Colon and rectum are formed from part of midgut and hindgut . Midgut forms ascending colon and proximal transverse colon. Hindgut forms distal transverse colon, descending colon , sigmoid colon , and rectum.

ANATOMY :

Large intestine is a hollow tube that make last 6 feet of the digestive tract. It consists of caecum , colon , rectum and anus.[5] Other organs including spleen , pancreas , liver, reproductive and urinary organs are next to colon and rectum . These organs will be affected if colorectal carcinoma spreads beyond the large intestine .

Colon consists of four parts

(i) Ascending colon – starts at caecum goes upward along right side and joins the transverse colon.

(ii) Transverse colon connects ascending colon and descending colon and is across the upper abdomen.

(iii) Descending colon – is between transverse colon and sigmoid colon and is on the left side of the body.

(iv) Sigmoid Colon - connects descending colon and rectum and is on the left side.

Hepatic flexure is the junction of ascending colon and transverse colon and splenic flexure is the junction of transverse colon and descending colon . Rectum is the distal 8- 15 cm of large bowel and it ends at anal canal.

Layers of colon and rectum :

Colon and rectum are made up of four layers . They are

- Mucosa
- Submucosa
- Muscularis propria
- Serous layer

1. MUCOSA :

It is the inner lining of intestine and it is formed by epithelium , lamina propria (connective tissue) and thin layer of muscle (Muscularis mucosa).

The epithelium of the mucosa is lined by single layer of cuboidal to low columnar cells in which is the opening of the crypts of LieberKuhn either into the grooves or on the surface.[6] The epithelium has two types of cells, the absorptive cells which have apical striated borders directed towards the lumen with acidophilic cytoplasm basally located nuclei . The other cell is the goblet cell which synthesize , store and secrete mucin granules . Crypts are tubular shaped and are parallel to one another . The crypts in addition to the absorptive cells and goblet cells also contain immature precursor cells , endocrine cells and Paneth cells . Paneth cells have many eosinophilic secretory granules that contain lysozyme , epidermal growth factor and other substances . Normally they are seen only in caecum and proximal right colon.

Lamina propria contains some lymphocytes, plasma cells, histiocytes and mast cells in a network of collagen fibers , smooth muscle bundles, vessels, and nerves. Lymphoglandular complexes

(microbursae) are structures which are normally seen extending from mucosa through mucularis mucosae into submucosa. They are formed by deep crypt epithelium which are surrounded by lymphoid follicles .

Muscularis mucosae is made up of thin layer of smooth muscle fibers and this separates submucosa from mucosa.

2. SUBMUCOSA :

It is made up of connective tissue , glands , lymphatic vessels, blood vessels and nerves.

3. MUSCULARIS PROPRIA :

It is the muscle layer made up of longitudinal outer layer and circular inner layer.

4. SEROUS LAYER :

It is made of single layer of flattened to cuboidal mesothelial cells and fibro elastic tissue .

Part of the colon is connected to the abdominal wall by mesentry. The upper two-third of rectum is covered by mesentry called mesorectum. The mesentry is made up of fatty connective tissue

that contain nerves, blood vessels, lymph nodes and lymphatic vessels that supply the colon.

NERVE SUPPLY:

Large intestine contains two types of nerve plexus. They are

- Meissner neural plexus

- Myenteric neural plexus of Auerbach

Submucosa contains the neural plexus of Meissner and Myenteric neural plexus of Auerbach is seen between the longitudinal and circular muscular layers.

BLOOD SUPPLY :

Caecum to splenic flexure is supplied by branches of superior mesenteric artery and distal to splenic flexure is by the inferior mesenteric artery. Branches of internal iliac arteries that is middle and inferior rectal arteries supply lower portion of rectum.

LYMPHATIC DRAINAGE :

The lymphatic vessels of ascending colon ends in ileocolic nodes and those of transverse colon end in right colic and middle colic nodes. All these end in superior mesenteric nodes. Lymphatic

vessels of descending colon reaches left colic nodes and those of sigmoid colon ends in sigmoid nodes. These nodes end in inferior mesenteric nodes. Lymphatic vessels from upper half of rectum reach pararectal nodes and ends in inferior mesenteric nodes . Lower half of lymphatic vessels reach internal iliac nodes.

FUNCTION:

Main functions of colon and rectum are to absorb nutrients and water from food and move food waste out of body by movement ie peristalsis .

EPIDEMIOLOGY :

Colorectal carcinoma peaks at 60 to 70 years of age. In a study by Nekalson et al (2008) showed that mean age of colorectal carcinoma was 55.7 years.[7] 20% of cases occur before 40 years and they usually have an inherited predisposition. Males are slightly more often affected than females. In a study by Dalal A.Elwy showed that 60% were males and 40% were females.[8] It is most prevalent in developed countries than the developing countries . According to U.S. SEER database , incidence rate for adenocarcinoma of colon is 33.7/100, 000 and that of rectal adenocarcinoma is 12.8/100, 000. Incidence and mortality have reduced in the last decade.

ETIOLOGICAL FACTORS :

NON MODIFIABLE RISK FACTORS :

1. Age

Diagnosis of colorectal cancer progressively increases from 40.[9]

2. Adenomatous polyps

Tubular adenomas and villous adenomas are precursor lesions of colorectal cancer. [10] Nearly 95% of sporadic colorectal cancers develop from these adenomas .[11] Long latency period of 5 to 10 years is required for occurrence of malignancy from these adenomas.[12]

3. Inflammatory Bowel disease

It includes two diseases

a) Ulcerative colitis which causes inflammation of mucosa of colon and rectum.

b) Crohn's disease causing inflammation of full thickness of bowel wall.

c) Risk of developing colorectal cancer in these conditions is estimated between 4 to 20 fold.[13]

4. Family history of Colorectal cancer or adenomatous polyp

Colorectal cancer develops in 20% of people who have family members with either colorectal cancer or adenomatous polyps.[14]

5. Genetic factors

Colorectal carcinoma is seen in association with the following inherited conditions.

a) Familial adenomatous polyposis

This occurs due to mutation in APC gene which is a tumour suppressor gene located on chromosome 5q21 and this has an autosomal dominant inheritance. In a study by Wilmink A BM it is seen that FAP accounts for <1% of colorectal cancers.[15]

b) Hereditary Non-polyposis colorectal cancer (Lynch syndrome)

Occurs due to mutation in MLH1 and MSH2 genes which are involved in DNA repair pathway.[16] Accounts for 2 % of colorectal cancers.

These mutations results in microsatellite instability. These colorectal carcinomas are usually associated with young age, involvement of proximal colon, mucinous features and tumour- infiltrating lymphocytes.

Other less common inherited syndromes associated with colorectal carcinoma are Peutz- Jegher's syndrome, Turcot syndrome,Cowden's syndrome and Torre-Muir syndrome.

MODIFIABLE LIFESTYLE RISK FACTORS :

1. Dietary factors :

Colorectal cancer occurs due to low intake of unabsorbable vegetable fibers and high intake of refined carbohydrates and fat. Reduced fiber content leads to decreased stool bulk and altered composition of intestinal microbiota which increase synthesis of toxic oxidative by products of bacterial metabolism which in contact with colonic mucosa cause damage. High fat intake enhances hepatic synthesis of cholesterol and bile acids which are converted by bacteria in the intestine to carcinogens.[17]

2. Physical inactivity and obesity:

Physical inactivity and excess body weight are associated with colorectal cancer.[18]

3. Smoking :

Cigarette smoking leads to the formation of adenomatous polyps which are the precursor lesions of colorectal cancer.[19] Those among who smoke, there is earlier age of onset of cancer.[20]

4. Alcohol consumption :

Alcohol consumption is associated with increased risk of developing colorectal cancer.In a study by Tsong WH et al it is seen that colorectal cancer in alcoholics is associated with a younger age.[21]

Mechanisms of developing colorectal carcinoma is that acetaldehyde and other reactive metabolites of alcohol may be carcinogenic. It also acts as a solvent for penetration of other carcinogens. It is also mediated by production of free radical oxygen species .[22]

5. Irradiation:

Therapeutic pelvic irradiation is associated with development of colorectal carcinoma.

PATHOGENESIS :

Two genetic pathways have been described.

1. APC/B-Catenin pathway which is associated with WNT and classic adenoma – carcinoma sequence.

2. Microsatellite instability pathway in which there is defects in DNA mismatch repair.

CLASSIC ADENOMA –CARCINOMA SEQUENCE

This accounts for 80% of sporadic colon tumours. Mutation of APC gene which is a tumour suppressor gene occurs early in the neoplastic process. Tumour occurs when both the copies are functionally inactivated. APC is a negative regulator of beta catenin which is a component of WNT signaling pathway. Beta catenin accumulates when there is loss of APC function and this translocates to nucleus and activates transcription of MYC and Cycle D1 genes which promote proliferation of cells.

Additional mutations are followed such as activating mutation of KRAS which promote growth and prevent apoptosis. Mutation of tumour suppressor genes SMAD2 and SMAD4 occurs which are effectors of

transforming growth factor beta signaling which normally inhibits cell cycle. Also there is loss of function of P53 which controls the cell cycle.

All these mutations occur due to chromosomal instability.

MICROSATELLITE INSTABILITY PATHWAY:

In DNA mismatch repair deficiency there is microstatellite instability ie mutation in micro statellite repeats. Some of these repeats are in the coding or promoter region of genes such as type II transforming growth factor beta receptor and pro-apoptotic protein BAX which are involved in regulation of cell growth. Transforming growth factor beta inhibits colonic cell proliferation and hence its mutation leading to uncontrolled cell growth and mutation in BAX leads to survival of genetically abnormal clones.

CLINICAL FEATURES

Colorectal cancers are seen insidiously and are asymptomatic in some patients. Common presenting features are hematochezia and anaemia.

Right sided colon cancers produce symptoms like fatigue and weakness due to iron deficiency anaemia. Left sided colon cancers produced symptoms like occult bleeding, change in bowel habits with

alternate constipation and diarrhoea, left quadrant discomfort or abdominal pain. Intestinal obstruction is commonly seen with left sided colon tumour. Rectosigmoid tumours produce tenesmus.

DIAGNOSTIC PROCEDURES

Imaging :

Early detection of colorectal carcinoma has increased over past few decades with the use of CT and screening studies in asymptomatic populations. CT is superior to conventional barium enema in detecting colorectal tumours. CT, MRI imaging and transrectal ultrosonography help in the assessment of depth and margins of the tumour and to detect regional and distant metastases. Scintigraphy and positron emission tomography are also used.[23]

TISSUE SAMPLING PROCEDURE :

Colonoscopic and sigmoidoscopic biopsy is the definite method for establishing a diagnosis of colon and rectal lesions

Indications of colonoscopy

1. Colorectal carcinoma screening in high risk people
2. Evaluation and removal of polyps

3. Current or previous bowel resection for colon cancer

4. Family history of cancer

5. Management of inflammatory bowel disease

6. Identification of acute bleeding sites

7. Decompression of colon

IMMUNOHISTOCHEMISTRY:

Immunohistochemistry is a adjuvant technique which is commonly used to diagnose colorectal tumours. Immunohistochemistry has largely replaced mucin histochemistry and electron microscopy in diagnosing tumours of colon and rectum because of relative ease of use and specificity.

Markers in colorectal carcinoma

The various immunohistochemical markers for colon carcinoma are

1. MUC1 and MUC3

2. P53

3. Cytokeratin 20

4. Carcino embryonic antigen

5. HER-2/ neu – Epidermal growth factor receptor

6. CDX2

7. TAG-72-Tumour associated glycoprotein

8. Cathepsin

9. Villin

10. HCG

11. PLAP- Placental Alkaline Phosphatase

MUC-1 and MUC-3

Mucins are glycoproteins which are expressed by many epithelial cells. They are also expressed in some of their malignant counterparts. Their expression can be used as prognostic indicator and also their use in cancer therapy.

There are several mucins such as MUC1, MUC2, MUC3, MUC4, MUC5 AC and MUC6. A study by Cao Y et al showed that MUC-1 and MUC3 are more commonly expressed in conventional colorectal carcinomas. [(24)] MUC2 expression is mostly seen in mucinous carcinomas. MUC13 expression is seen particularly in poorly differentiated tumours.

Mucins are also expressed in many other malignancies such as breast carcinoma, pancreatic and gastric carcinoma. In colorectal carcinoma there is usually no expression of MUC5AC which is commonly expressed in pancreato biliary tumours.

CYTOKEROTIN 20

Cytokeratin 20 is a protein found in mature enterocytes and goblet cells and it is a protein which is specifically found in gastric and intestinal mucosa .[25] It is encoded by the gene KRT20 in humans.[26] Colorectal adenocarcinomas are positive for cytokeratin 20 invariably.[27]

Positivity with CK 20 is also found in transitional cell carcinomas and in Merkel cell carcinoma. Positivity with cytokeratin 20 and negativity for cytokeratin 7 is the most common pattern and is used to differentiate colorectal adenocarcinomas from adenocarcinomas of lung, ovary and prostate.[28]

CARCINOEMBROYONIC ANTIGEN :

Carcinoembroyonic antigen are glycosyl phosphatidyl inositol cell surface anchored glycoprotein involved in cell adhesion.It is normally produced during fetal development in gastro intestinal tissue and its production stops before birth.

But in certain cancers such as colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, lung carcinoma, breast carcinoma, medullary thyroid carcinoma and in certain non neoplastic conditions like ulcerative colitis, pancreatitis , cirrhosis, COPD, Crohn's disease and hypothyroidism expression of CEA and its serum levels are increased.[29] And therefore it is used as tumour marker in these tumours.

HER-2/ NEU

Human epidermal growth factor receptors are G-protein receptors which when activated stimulate multiple signal transduction pathways which are involved in regulation of cellular growth. HER 1(EGFr), HER2(Her-2neu or ERB2),HER3 and HER4 are the HER family of receptors. Of these HER- 2 amplification and overexpression has been seen in certain tumours like breast cancer, colorectal cancer, oesophageal carcinoma, gastric carcinoma and ovarian tumours.

HER - 2 is located on chromosome 17q21. It encodes a 185KD transmembrane protein. Its activation initiates pathways that are important for cell proliferation and differentiation such as MAPK- Mitogen activated protein Kinase and P13K/AK7 (3 Kinase) pathways. In recent years this protein is used an important tumour marker and is also used as a target of therapy.

P53

P53 is a tumour suppressor protein encoded by TP53 gene located on short arm of chromosome 17.[30] The function of P53 protein is that it can arrest cell cycle at G1/S regulation point when it recognizes DNA damage and its activates DNA repair protein which help to correct the damage. If DNA damage is irreparable it will initiate apoptosis of the affected cell. It has been found that TP 53 gene is most frequently mutated gene in most human cancers.

Colorectal carcinomas commonly involves P53 mutation which leads to mutated P53 protein expression in tumour cells. Its expression is found to be associated with poor prognosis and reduced survival.

CDX2

CDX2 is a homeobox gene which encodes a transcription factor that takes part in proliferation and differentiation of intestinal epithelial cells. It is found to be expressed in majority of colorectal adenocarcinoma.[31] In a study by Mazziotta RM et al showed that CDX2 was also expressed in mucin producing carcinomas of ovary, lung, bladder and pancreatobiliary adenocarcinomas.[32]

TAG-72

Tumour associated glycoprotein 72 is a glycoprotein found on surface of many cancer cells including breast, ovary, colon, and pancreatic cells.[33] It is present in 100% of invasive colorectal tumours. It is also found in hyperplastic and adenomatous polyp and also in normal mucosa. The pattern of expression and frequency of reactivity vary depending on the conditions.[34]

CATHEPSIN B

Cathepsin B is a lysosomal cysteine protease coded by the CTSB gene.[35] It is found expressed in colorectal carcinomas and in wide array of disease.

VILLIN

Villin is a actin-binding protein found associated with actin core bundle of brush border of microvilli.[36] It is expressed in colorectal adenocarnimas .

HUMAN CHORIONIC GONADOTROPIN

Human Chorionic gonadotropin is a hormone produced by syncytio trophoblast. It is elevated in some cancerous conditions.

Colorectal adenocarcinomas show increased expression of HCG and mostly seen in mucinous and poorly differentiated tumours.[37]

PLACENTAL ALKALINE PHOSPHATASE

Placental alkaline phosphatase is an enzyme encoded by ALPP gene.[38] PLAP is a tumour marker for seminoma and ovarian cancer. Also seen in 10% of Colorectal carcinoma.

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF COLON AND RECTUM

EPITHELIAL TUMOURS

Adenoma 8140/0

- Tubular 8211/0
- Villous 8261/0
- Tubulovillous 8263/0
- Serrated 8213/0

Intraepithelial neoplasia (dysplasia)

Associated with chronic inflammatory diseases

- Low-grade glandular intraepithelial neoplasia
- High-grade glandular intraepithelial neoplasia

Carcinoma

- Adenocarcinoma 8140/3
- Mucinous adenocarcinoma 8480/3
- Signet-ring cell carcinoma 8490/3
- Small cell carcinoma 8041/3
- Squamous cell carcinoma 8070/3
- Adenosquamous carcinoma 8560/3
- Medullary carcinoma 8510/3
- Undifferentiated carcinoma 8020/3

Carcinoid (well differentiated endocrine neoplasm) 8240/3

- EC-cell, serotonin-producing neoplasm 8241/3
- L-cell, glucagon-like peptide and PP/PYY producing tumour
- Others

Mixed carcinoid-adenocarcinoma 8244/3

Others

NON-EPITHELIAL TUMOURS

- Lipoma 8850/0
- Leiomyoma 8890/0
- Gastrointestinal stromal tumour 8936/1
- Leiomyosarcoma 8890/3
- Angiosarcoma 9120/3
- Kaposi sarcoma 9140/3
- Malignant melanoma 8720/3
- Others

Malignant lymphomas

- Marginal zone B-cell lymphoma of MALT Type 9699/3
- Mantle cell lymphoma 9673/3
- Diffuse large B-cell lymphoma 9680/3
- Burkitt lymphoma 9687/3
- Burkitt-like /atypical Burkitt-lymphoma 9687/3
- Others

SECONDARY TUMOURS

Polyps

Hyperplastic (metaplastic)

Peutz-Jeghers

Juvenile

PRECURSOR LESIONS :

1. Aberrant crypt foci
2. Adenomas
3. Hyperplastic polyps
4. Juvenile polyp
5. Inflammatory polyps
6. Chronic inflammatory bowel disease

Aberrant crypt foci:

Aberrant crypt foci are the earliest morphological precursor of epithelial neoplasia. Microscopically they have thickened epithelium with enlarged calibre and reduced mucin content.

There are two main types

1. Aberrant crypt foci with features of hyperplastic polyps.

   RAS protooncogene mutations are seen in high frequency.

2. Dysplastic Aberrant crypt foci (Micro-adenomas)

   Mutation of APC gene is seen

Carcinogenesis in large intestine is characterized by progression from ACF through adenoma to carcinoma.[39] This was also observed in a study by Nascimbeni R et al.[40]

Adenomas :

Adenomas are precursor lesions defined by the presence of intraepithelial neoplasia. It is histologically characterized by increase in cellularity, enlarged hyper chromatic nuclei, nuclear stratification and loss of polarity. They can be low grade or high grade which depends on degree of villous or glandular complexity, extent of nuclear stratification, and severity of nuclear morphology.

Macroscopically adenomas are classified into three groups.

1. Elevated adenoma - ranges from pedunculated to sessile adenomas.

2. Flat adenomas

3. Depressed adenomas

Histopathologically they are divided into

1. Tubular adenomas

2. Villous adenomas

3. Tubulo villous adenomas

Tubular adenomas

They are usually spherical and pedunculated or flat lesions.

Microscopically 80% of luminal surface is occupied by dysplastic glandular structures.

Villous adenomas

They are typically sessile with hairy appearing surface.

Microscopically 80% of luminal surface is lined by leaf like projections lined by dysplastic glandular epithelium.

Tubolovillous adenoma

Mixture of both tubular and villous structures are seen.

Serrated Adenomas:

Serrated Adenomas are hyperplastic polyp characterized by saw-tooth configuration. The upper portion of crypts and luminal surface are lined by epithelium having dysplastic changes.

Hyperplastic Polyps

Hyperplastic polyps is a mucosal excrescence characterized by elongated, crypts which are lined by proliferative epithelium in the bases. It has a saw tooth outline due to infolded epithelial tufts and enlarged goblet cells in the upper crypts and on luminal surface. The nuclei of epithelial cells are small regular round and located at base of cells with cytoplasm having prominent mucin.

Juvenile Polyps

These are usually seen in children. They can occur either sporadically or genetically. Sporadic juvenile polyps are spherical, lobulated and pedunculated. Microscopically they have abundant stroma composed of granulation tissue which is inflamed and oedematous and it surrounds cystically dilated glands lined by cuboidal to columnar cells with reactive changes and they contain mucin. Polyps in juvenile polyposis syndrome have frond like pattern with more proliferative small glands and few dilated glands and stroma.

Inflammatory polyps :

These polyps have reactive epithelium, inflamed granulation tissue and fibrous tissue in varying proportions. These polyps are seen in chronic inflammatory bowel disease and diverticulitis.

Chronic inflammatory bowel disease :

Chronic colitis is associated with colorectal carcinomas .Chronic colitis is seen in ulcerative colits and crohns disease.

ADENOCARCINOMA

Conventional adenocarcinomas account for more than 90% of colorectal carcinomas. Sigmoid and rectum are most commonly affected sites. In a study by Slater G etal it was seen that in elderly patients and in diverticular disease right sided tumours are most common.[41]

Macroscopy:

Macroscopically colorectal carcinomas can be of the following types:

a. Exophytic/fungating -Intraluminal growth predominantly. Exophytic masses are usually seen in proximal colon carcinomas.

b. Endophytic/ ulcerative –Intramural growth predominantly

c. Diffusely infiltrative / linitis plastica

d. Annular – circumferential involvement of colorectal wall and leads to constriction of lumen.

Endophytic masses and annular growth as usually seen in carcinomas of transverse colon and descending colon.

Cut section:

On cutsection colorectal carcinomas have homogeneous grayish white tissue that replaces the bowel wall. Areas of necrosis can be seen. Tumour is seen either confined to the wall or extended into pericolic tissues.

Microscopy :

Conventional adenocarcinomas is characterized by glandular formation. Depending on the gland formation tumour has been classified into following types.

a. Well-differentiated adenocarcinoma – gland formation is seen in more than 95% of the tumour.

b. Moderately differentiated adenocarcinoma – 50-95% of the tumour shows gland formation.

c. Poorly differentiated adenocarcinoma <50% of the tumour shows gland formation.

Most common colorectal adenocarinomas are well to moderately differentiated adenocarcinomas.Well differentiated tumour show well formed glands lined by tall columnar cells with abundant eosinophilic cytoplasm and basally located nuclei with mild atypia. Mitotic figures are less frequent.

Moderately differentiated tumours show haphazardly arranged imperfectly formed glands lined by tumour cells showing moderate atypia. Mitotic figures are variable.

Poorly differentiated tumours show solid sheets of tumour cells which show marked atypia. Mitosis is more frequent.

Colorectal adenocarcinomas are said to be invasive when they invade through muscularis mucosae into submucosa. Other features of invasiveness are presence of desmoplasia or demoplastic reaction which is a type of fibrous proliferation seen surrounding the tumour cells and the presence of necrotic debris in glandular lumina called dirty necrosis.

Variants:

1. Mucinous adenocarcinoma

Mucinous adenocarcinomas constitute of about 15% of colorectal carcinomas. They are most commonly seen in the rectum. Macroscopically they usually have an exophytic growth with grossly visible mucin. Only when more than 50% of the tumour is composed of mucin it is called as mucinous adenocarcinomas. When the mucinous component is >10% but <50% it is termed as adenocarcinoma with mucinous features or differentiation.

Tumour is characterized by pools of extracellular mucin that contain malignant epithelial cells as acinar structures, strips of cells or single cells. Mucinous adenocarcinoma shows high rate of microsatellite instability. Prognosis of mucinous adenocarcinoma is poor when compared with conventional adenocarcinoma.

Signet ring carcinoma:

Signet ring carcinoma is also called as Linitis Plastica-type carcinoma. Signet ring carcinoma is a rare variant of colorectal carcinoma. It is commonly seen in young patients. Grossly it most commonly presents as diffuse infiltration of bowel wall but also arise in adenomatous polyp.[42][43]

Microscopically cells may be arranged in diffuse sheets, nests or infiltrative cords. The signet ring cell has a large mucin vacuole that fills the cytoplasm and displaces and compresses the nuclei to the periphery.

Signet ring cell carcinoma is defined only when more than 50% of cells in the tumour have intracytoplamic mucin.[44] These cells can occur in diffuse infiltrative pattern with minimal extra cellular mucin or in pools of mucin in mucinous adenocarcinoma. Metastasis occur in lymphnode, ovary and peritoneal surface. Prognosis is poor.[45]

Micro papillary variant:

This is seen in 20% of colorectal adenocarcinomas. It is associated with increased frequency of lymphovascular invasion and lymph node metastases and therefore it has poor prognosis.[46]

Serrated adenocarcinoma:

Serrated adenocarcinomas comprise 7.5% of all colorectal tumours. It is presumed to arise from serrated adenoma.[47] Pattern of growth seen is serrated, mucinous or trabecular. Cells have abundant eosinophilic cytoplasm, chromatin condensation with preserved polarity.[48]

Adenosquamous carcinoma:

These are rare tumours showing both squamous carcinoma and adenocarcinoma features. They can be seen either as admixed within the tumour or can be seen as separate areas. This carcinoma can be seen anywhere in the large bowel but more common in the caecum.[49][50] The squamous differentiation must be more than just occasional small foci for the tumour to be classified as adenosquamous. Pure squamous cell carcinoma is very rare.

Medullary carcinoma:

This rare variant is mostly seen in right colon and is more common in females. This is characterized by malignant cells arranged in sheets having abundant eosinophilic cytoplasm with vesicular nuclei and prominent nucleoli. It exhibits prominent intraepithelial lymphocytic infiltration.[51] They have a favourable prognosis.

Other rare variants:

Spindle cell or sarcomatoid variant:

This variant is composed of spindle cells and are focally immunoreactive for cytokeratin.

Carcinosarcoma :

This contains both carcinomatous and heterologous mesenchymal elements.

Clear cell variant:

They have clear cytoplasm due to accumulation of glycogen.[52]

Anaplastic (Pleomorphic, giant cell) variant

Trophoblastic variant[53]

Basaloid variant[54]

Hepatoid differentiation

Glassy Cell variant[55]

Rhabdoid variant[56]

Oncocytic variant

Neuroendocrine differentiation

This can occur in the following ways.

a) As scattered endocrine cells in a mucinous adenocarcinoma.In a study Bosman FT also found these cells scattered in mucinous

adenocarcinoma.[57] This occurs most commonly after chemotherapy or radiotherapy and it does not affect the prognosis .

b) As mixed composition with both adenocarcinomatous component and endocrine differentiation.[58]

c) Occurs with predominantly neuroendocrine differentiation which can be either large cell or small cell neuroendocrine carcinoma.

STAGING :

There are many staging systems for colorectal carcinoma.

- Duke's Classification
- Staging system of Astler and Collar
- Staging system by American Joint Commmittee
- TNM Classification of staging

1. Duke's Classification

This classification was proposed on 1937.[59] This staging method is simplest and is directly related to prognosis.

Stage A – only the wall of the bowel is involved by the tumour.

Stage B - Tumour extends through the bowel wall.

Stage C - Tumours having lymphnode metastases.

Modificaitons were proposed and further subclassified stage-C to C1 and C2 and also included stage D.

Stage C1- Where only regional lymph nodes were involved.

Stage C2- Involvement of nodes at mesenteric blood vessels.[60]

Stage D - which indicates distant metastasis.

2. Astler and Coller classification

This classification was proposed in 1954.[61]

Stage A - Tumour limited to mucosa.

Stage B1 - Tumour involves muscularis externa but does not penetrate through it.

Stage B2 - Tumour penetrates through muscularis externa.

Stage C1 - Tumour is confined to bowel wall with nodal metastases.

Stage C2 - Tumour penetrates through the bowel wall with nodal metastases.

3. TNM Classification :

This classification now seems to replace Duke's Classification.[62]

T – Primary Tumour

TX Primary tumour cannot be assessed

T0 No evidence of primary tumour

Tis Carcinoma in situ: intraepithelial or invasion of lamina propria

T1 Tumour invades submucosa

T2 Tumour invades muscularis propria

T3 Tumour invades through muscularis propria into subserosa or into non-peritonealized pericolic or perirectal tissues

T4 Tumour directly invades other organs or structuresand/or perforates visceral peritoneum

N – Regional Lymph Nodes

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in 1 to 3 regional lymph nodes

N2 Metastasis in 4 or more regional lymph nodes

M – Distant Metastasis

MX Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis

Stage Grouping

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
	T2	N0	M0
Stage II	T3	N0	M0
	T4	N0	M0
Stage III	Any T	N1	M0
	Any T	N2	M0
Stage IV	Any T	Any N	M1

SPREAD AND METASTASIS :

The tumour can spread in the following ways :

1. Direct spread

2. Lymphatic spread

3. Hematogenous spread

Direct Spread :

Tumour involve contiguous structures by direct extension through muscularis propria into pericolic or perirectal soft tissues.

Depending upon the anatomic site will be the consequences of direct extension.

LYMPHATIC SPREAD :

Regional lymph nodes are the common site of metastatic involvement. Lymphatic spread occurs only when muscularis mucosae is breached and when there is involvement of submucosa eventhough there is presence of lymphatics in the mucosa. Lymph node metastases has great prognostic significance and hence there must be thorough search for lymphnodes. Minimum number of 14 or 15 nodes must be recovered from surgical specimen of colorectal carcinoma.[63] Pericolonic tumour

deposits are isolated tumour nodules. These are seen beyond muscularis propria in perineurial, perivascular or intravascular location. These must be distinguished from lymph node metastasis.[64]

HEMATOGENEOUS SPREAD:

Hematogeneous spread occurs through invasion of tributaries of portal vein in colon and tributaries of vena cava in rectum. Most common site of metastasis is liver. In a study by Inada K et al liver was found to be common site of metastasis.[65] Peritoneum, lung and ovaries are other metastatic sites which are relatively common.

Central nervous system, bone, testis, uterus and oral cavity are metastatic sites which are rare.[66][67]

GRADING

Depending on the extent of glandular formation, tumours are graded.

Grade I -- Well differentiated

Grade II -- Moderately differentiated

Grade III -- Poorly differentiated

Grade IV -- Undifferentiated

Mucinous and signet ring carcinomas are considered as grade III (Poorly differentiated grade). Medullary carcinoma is considered as grade IV (undifferentiated grade).

Grading can also be done as

Low-grade which includes well and moderately differentiated carcinomas. High grade which includes poorly differentiated and undifferentiated tumours.

TREATMENT

Surgical resection is the standard therapy for colorectal carcinomas. Depending on the site of tumour is the type of surgery.[68] Ileocolectomy is done for carcinoma of caecum or ascending colon. Abdominoperineal resection done for tumours below peritoneal reflection.[69] Anterior resection is done for tumours in other areas of large bowel. For small rectal tumours or those who are not fit for abdonimoperineal resection other alternative procedures such as fulguration, endoscopic transrectal resection and full thickness local excision are done.[70][71]

PROGNOSIS

Number of clinical and pathologic parameters determine the prognosis of colorectal carcinoma.[72]

AGE :

Age has important role in rectal tumours than colonic tumours. Very young and very old patients have poor prognosis.[73]

SEX:

Females have better prognosis than males.

CEA SERUM LEVELS:

Adverse impact on prognosis is seen when CEA levels are more than 5ng/ml.[74]

TUMOUR LOCATION:

Tumours have favourable prognosis when they are situated in left colon. Worst outcome is seen in tumours located at sigmoid colon and rectum.[75]

TUMOUR SIZE:

Both large and small tumour size have poor prognosis.[76]

TUMOUR EDGE :

Polypoid tumours have good prognosis when compared with non-polypoidal tumours.

TUMOUR MARGIN AND INFLAMMATORY REACTION :

Tumours having pushing margins have better prognosis. Presence of inflammatory infiltrate at the interphase between tumour and nearby tissue has better prognosis.

LOCAL EXTENT :

Tumours restricted to mucosa and submucosa have good prognosis whereas tumour involving the whole bowel wall or perforation have worse prognosis.

TUMOUR BUDDING :

At the invasive front if there are isolated tumour cells or clusters of more than 5 cells indicates poor outcome.[77]

VASCULAR INVASION :

When there is presence of vascular invasion the incidence of distant metastasis is increased and hence the survival is reduced thus indicating poor prognosis. Tumour involving the extramural blood

vessels is of more prognostic significance than when it involves the vessels within the bowel wall.

PERINEURIAL INVASION :

This indicates advanced disease and has worst prognosis.

PERICOLONIC TUMOUR DEPOSITS :

Presence of pericolonic tumour deposits have poor prognosis.[78]

SURGICAL MARGIN :

In rectal carcinoma involvement of radial margin indicates poor prognosis and also predicts local recurrence.

MICROSCOPIC TYPE AND PATTERN :

Medullary carcinoma has good prognosis. Mucinous carcinoma, signet ring carcinoma and anaplastic carcinoma have worst prognosis. Microacinar pattern has worse prognosis.

GENETIC FACTORS :

Tumours have poor prognosis when there is expression of mucin related antigens such as sialyl-TN and sialyl-Lewis (x), expression of fascin, expression of PR6 and P16INK4.

Tumours with allelic loss of chromosome 18q and loss of claudin-1 (tight junction –associated protein) have poor prognosis.[79][80] Tumours with HLA-DR expression, Bcl-2 protein expression and TGF-beta1 mutations have favourable outcome.[81][82]

LYMPH NODE INVOLVEMENT :

Lymph node involvement has a decreased survival rate. When nodes involved are other than those nodes which are close to the tumour indicates poor prognosis.[83] Worse is the prognosis when more number of lymph nodes are involved .

PATTERN OF LYMPH NODE REACTION :

When there are reactive changes produced by cell-mediated immune response it indicates good prognosis.

STAGING AND GRADING :

Staging and grading are very important predictors for prognosis.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN :

Prospective study.

PLACE OF STUDY :

Department of Pathology, Coimbatore Medical College Hospital, Coimbatore.

STUDY PERIOD :

July 2014 – July 2015

INCLUSION CRITERIA :

1. All resected colorectal specimens received in Department of Pathology
2. Well fixed adequate tissues.

EXCLUSION CRITERIA

1. Inappropriately fixed specimens.

Sections of four micron thickness were cut on to coated slides . The slides are then incubated at 58 degrees overnight. The initial sections which were cut were stained with hematoxylin and eosin stain.

METHOD OF HEMATOXYLIN AND EOSIN STAINING

REAGENTS USED:

1. Erhlich's Hematoxylin solution

2. 1% Eosin Y solution

3. 1% Acid alcohol solution

PROCEDURE

1. Sections deparaffinized in xylene by immersing for 30 seconds.
2. Sections are then placed in Isopropyl alcohol for 15 minutes.
3. Wash in running tap water.
4. Stain the sections for 10 to 15 minutes with Erhlich's Hematoxylin solution.
5. Wash in running tap water.
6. Differentiate with acid alcohol 1% solution-two to three dips.
7. Blueing is done for 10 minutes.
8. Counterstain is done with eosin 1 % solution – 3 to 4 dips.
9. Wash in tap water.
10. Sections are air-dried.
11. Dip in xylene and mount in DPX.

The sections stained with hematoxylin and eosin are studied and based on the histomorphological features, a diagnosis is obtained.

IMMUNOHISTOCHEMISTRY :

METHOD :

Two Step indirect technique.

PRINCIPLES OF IMMUNOHISTOCHMISTRY :

The principle of this technique is detection of antigens expressed in the cells and tissues and is done with two step process.

1. Primary antibody using specific epitopes is bound to antigens.
2. The antigen-antibody binding is then detected by calorimetric reaction.

REAGENTS :

1. Peroxide Block : 3% hydrogen peroxide in water.
2. Power block: contains casein and properity additives with 15mM sodium azide in PBS. It is a protein blocking reagent and it is highly effective.
3. Chromogen : DAB-33' diaminobenzidine.

4. Liquid DAB substrate : Tris buffer which contains peroxides and stabilizers.

5. Super enhancer reagent.

6. Counterstain with Meyer's Hematoxylin.

7. Buffer solutions

CITRATE BUFFER (pH 6.0)

Trisodium Citrate : 2.94 grams

Distilled water : 1000 ml

IN HCL : 5 ml

TRIS EDTA : (pH 9.0)

TRIS buffer salt : 6.05 grams

Disodium EDTA : 0.744 grams

Distilled water : 1000ml

TRIS BUFFER(pH 7.6)

TRIS buffer salt : 0.605 grams

Sodium Chloride : 8 grams

Distilled water : 1000 ml

IN HCL : 3 ml

PROCEDURE :

1. Sections are deparaffinized for 30 minutes in xylene

2. Absolute alcohol for 5 minutes with two changes

3. Wash in running tap water for 10 minutes

4. Rinse for 5 minutes in distilled water

5. Antigen retrieval is done in microwave by keeping the slides in it with appropriate buffer solutions

   Medium : 10 minutes and high-10 minutes

6. Cool the sections to room temperature

7. Rinse in distilled water

8. TBS buffer wash for 5 minutes with 2 changes

9. Peroxide block for 10 minutes

10. TBS buffer wash for 5 minutes with 2 changes

11. Treat with power block for 10 minutes

12. Cover the sections with primary antibody (supplied from DAKOCYTOMATION)

13. TBS buffer wash for 5 minutes with 2 changes

14. Super enhancer for 30 minutes

15. TBS buffer wash – 5 minutes 2 changes

16. Poly HRP reagent for 30 minutes

17. TBS Buffer wash for 5 minutes with 2 changes

18. DAB Chromogen and substrate buffer for 5 to 8 minutes.

19. TBS Buffer wash for 5 minutes -2 changes

20. Wash in running tap water for 5 minutes

21. Counterstain for 1 minute with Mayers Hematoxylin

22. Tap water wash for 5 minutes

23. Air dry and mount in DPX

Positive Control for HER-2/neu includes breast carcinoma slides that is HER-2/neu immunoreactive. Negative control was also run each time.

Immunohistochemical Evaluation

Sections are examined under high power objective for positive immunoreactivity. Positivity is considered when the neoplastic cells have

cytoplasmic, membranous or membranous and cytoplasmic golden brown staining. Semiquantitative scoring was carried out.

Grading of intensity of staining

0 - Absent

1 - Weak

2 - Moderate

3 - Strong

Percentage of positive tumour cells:

+1 - 10-40% of cells are positive

+2 - 41-70% of cells are positive

+3 - >70 % of cells are positive.

HER-2/neu was considered positive if >10% of cells have strong immunostaining or >50% of cells show moderate staining.

Data :

Tumour was subtyped using H & E sections according to WHO classification. Patient age, gender and HER-2/neu status was determined and expressed.

Statistical analysis :

For analysis SPSS software version was used and the variables expressed as percentage of number (%). For statistical comparisons Chisquare tests was employed. p Value <0.05 was considered statistically significant.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

The present study is a prospective study conducted in the Department of Pathology, Coimbatore Medical college Hospital. A total of 30 cases of colorectal adenocarcinoma specimens received over the period of July 2014 to July 2015 were studied.

Ethical clearance was obtained from Ethics committee of Coimbatore Medical College and Hospital,Coimbatore.

Histomorphological and immunohistochemical pattern of expression were studied,analysed and compared with the literature.

TABLE 1

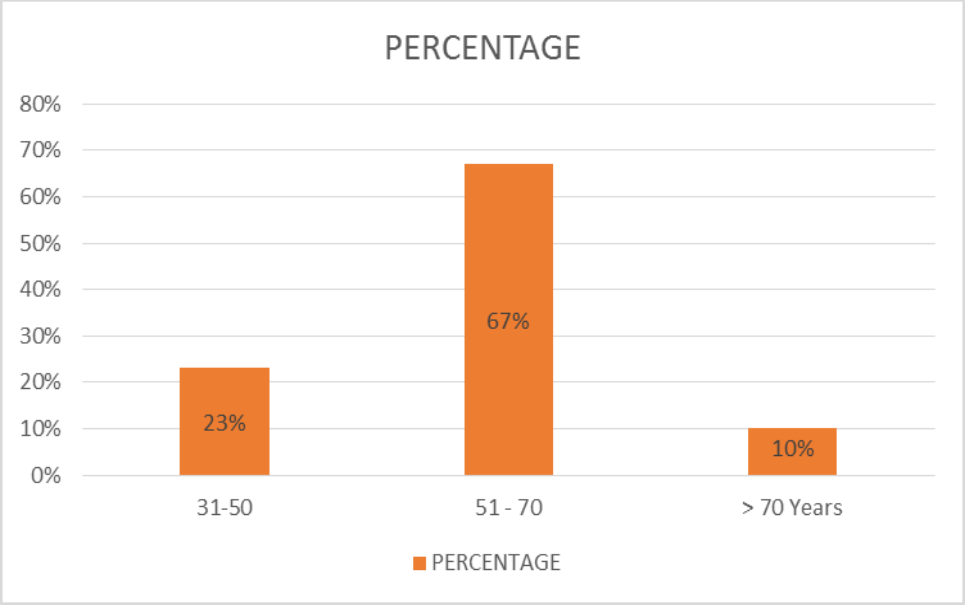
AGE WISE DISTRIBUTION OF COLORECTAL ADENOCARCINOMAS (n=30)

AGE	CASES	PERCENTAGE
31-50	7	23%
51 – 70	20	67%
> 70 Yrs	3	10%
Total	30	100%

In the above frequency table, it was observed that the incidence of colorectal carcinomas was high (67%) in the age group of 51-70 years, followed by the age group of 31-50 years (23%), and >70 years (10%). Patients age ranges from 34-80 years. Mean age is 58.6+/-24.6 years.

CHART 1

AGE DISTRIBUTION OF COLORECTAL ADENOCARCINOMAS (n=30)



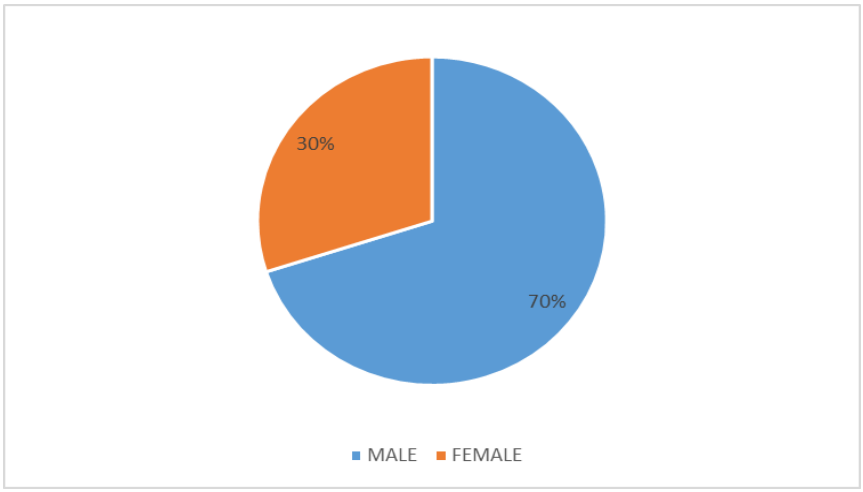
From the above bar chart it is observed that the incidence of colorectal carcinomas was high (67%) in the age group of 51-70 years, followed by the age group of 31-50 years (23%), and >70 years (10%).

TABLE 2

SEX WISE DISTRIBUTION OF COLORECTAL ADENOCARCINOMA(n=30)

SEX	CASES	PERCENTAGE
MALE	21	70%
FEMALE	9	30%
Total	30	100%

CHART 2



It was observed in the present study that the incidence of colorectal malignancy was comparatively high in males (70%) than in females (30%), with a male to female ratio of 2.3: 1

TABLE 3

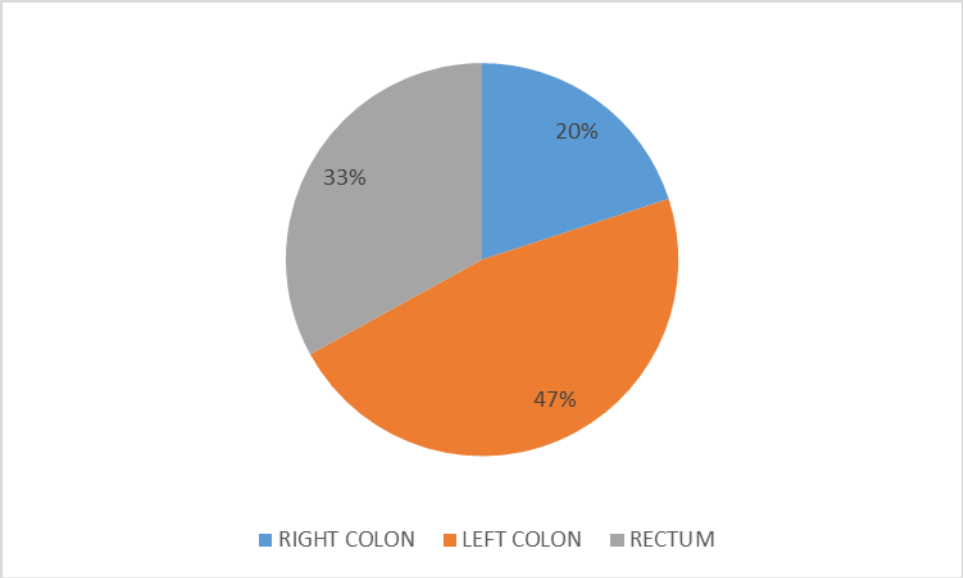
ANATOMICAL SITEWISE DISTRIBUTION OF COLORECTAL MALIGNANCY (n=30)

LOCATION	CASES	PERCENTAGE
RIGHT COLON	6	20%
LEFT COLON	14	47%
RECTUM	10	33%
Total	30	100%

It was observed from the above frequency table, among 30 cases studied the incidence of colorectal malignancy was comparatively high in left colon (47%) followed by rectum (33%) and right colon (20%).

CHART 3

ANATOMICAL SITEWISE DISTRIBUTION OF COLORECTAL MALIGNANCY (n=30)



From the above pie diagram it is observed that colorectal malignancy was comparatively high in left colon (47%) followed by rectum (33%) and right colon (20%).

TABLE 4

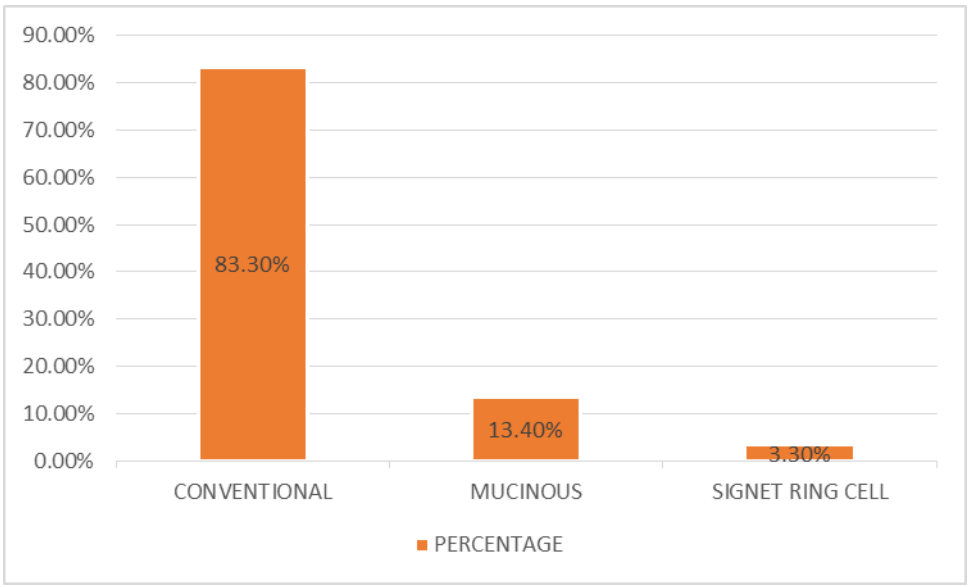
INCIDENCE OF VARIOUS SUBTYPES OF COLORECTAL ADENOCARCINOMA (n=30)

TYPES	CASES	PERCENTAGE
CONVENTIONAL	25	83.30%
MUCINOUS	4	13.40%
SIGNET RING CELL	1	3.30%
Total	30	100%

It is observed from the above frequency table that conventional adenocarcinoma was 83.3% followed by mucinous adenocarcinoma 13.4% and signet ring cell carcinoma 3.3%.

CHART 4

INCIDENCE OF VARIOUS SUBTYPES OF COLORECTAL ADENOCARCINOMA (n=30)



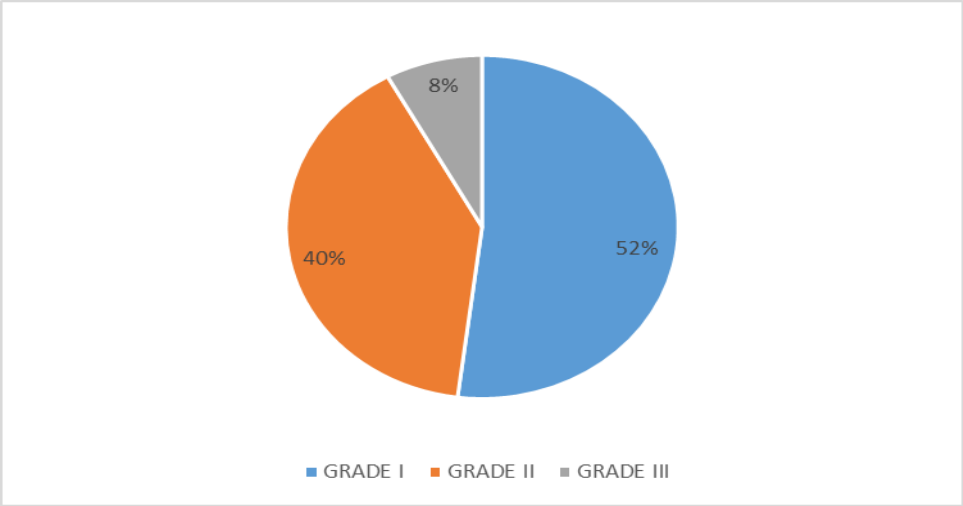
In the present study among the many subtypes in colorectal adenocarcinoma only three subtypes was observed. Among the three subtypes, conventional adenocarcinoma (83.3%) was most common followed by mucinous adenocarcinoma (13.4%) and signet ring cell carcinoma (3.3%).

TABLE 5

INCIDENCE OF GRADES OF CONVENTIONAL ADENOCARCINOMA (n=25)

GRADE	CASES	PERCENTAGE
GRADE I	13	52%
GRADE II	10	40%
GRADE III	2	8%
Total	25	100%

CHART 5



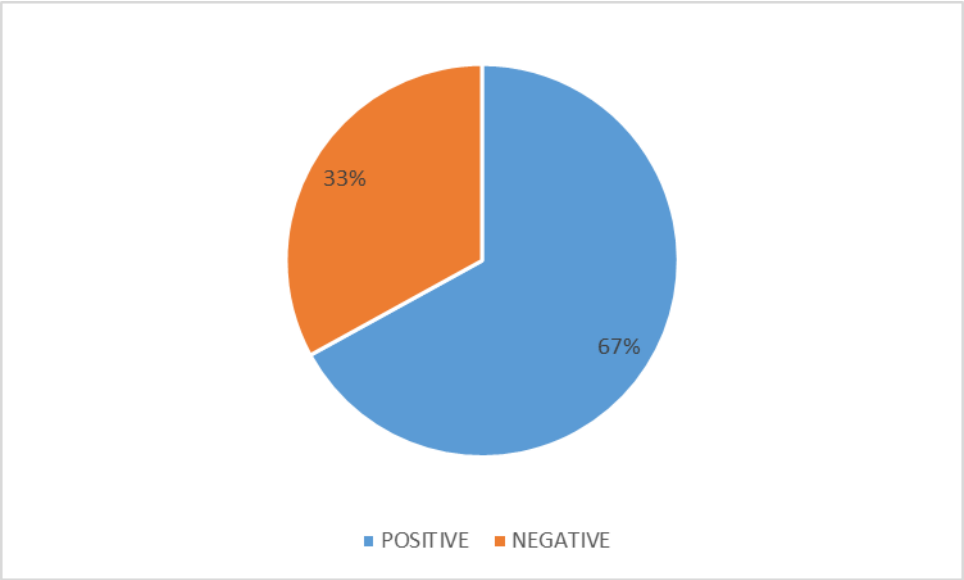
It was observed that among twenty five cases of conventional adenocarcinoma, well differentiated (grade I) was 52%, moderately differentiated (grade II) was 40% and poorly differentiated (grade III) was 8%.

TABLE 6

HER-2/neu POSTIVITY IN COLORECTAL ADENOCARCINOMAS (n=30)

HER2/neu	CASES	PERCENTAGE
POSITIVE	20	67%
NEGATIVE	10	33%
Total	30	100%

CHART 6



In the present study it was observed that HER-2/neu positive cases are 20 (67%) and negative cases are 10 (33%).

TABLE 7

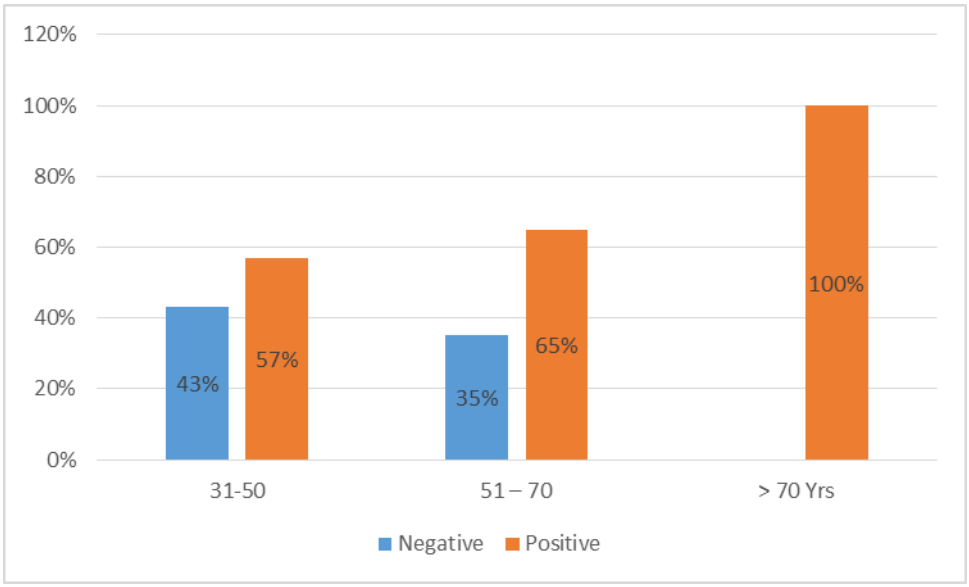
AGE WISE DISTRIBUTION OF HER-2/ neu EXPRESSION

HER2/neu					
AGE	**-VE**	**+1**	**+2**	**+3**	**+VE (%)**
31-50	3 (43%)	2	0	2	57%
51 – 70	7 (35%)	4	3	6	65%
> 70 Yrs	0	1	0	2	100%
Total	10	7	3	10	

In the current study out of 30 cases 7 cases were seen in the age group of 31-50 years of which 4 (57%) were HER-2/neu positive and 3 (43%) were negative. 20 cases were in the age group of 51-70 years of which 13(65%) were HER-2/neu positive and 7(35%) were negative. 3 cases were in the age group of > 70 years of which all the 3(100%) were HER-2/neu positive. These results were analysed using Chi-square analysis and found to be statistically significant with a p value of 0.03.

CHART 7

AGE WISE DISTRIBUTION OF HER-2/ neu EXPRESSION



In this clustered column diagram it is seen that HER-2/neu positivity was higher in age group >70 years in which all the 3(100%) cases showing positivity followed by 51-70 years showing 65% positivity and 31-50 years showing 57% positivity. These results were analysed using chi-square analysis and found to be statistically significant.

TABLE 8

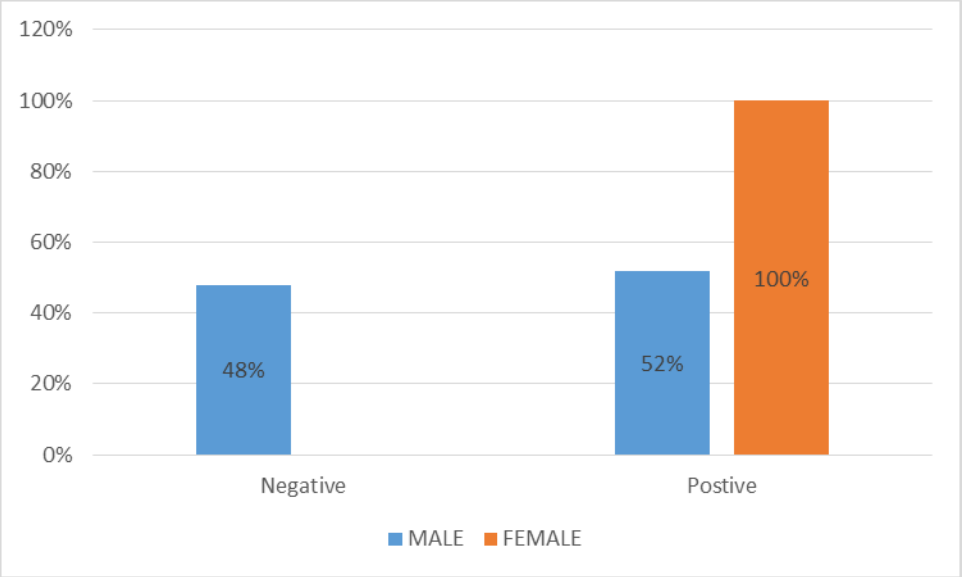
SEX WISE DISTRIBUTION OF HER-2/ neu EXPRESSION

	HER2/neu				
SEX	**-VE**	**+1**	**+2**	**+3**	**+VE(%)**
MALE	10 (48%)	4	3	4	52%
FEMALE	0	3	0	6	100%
Total	10	7	3	10	

In the present study out of 21 males,11 cases (52%) were HER-2/neu positive and 10 cases were HER-2/neu negative. Whereas among 9 females all of them(100%) were HER-2/neu positive.Chi square analysis showed a p value < 0.05 which is statistically significant.

CHART 8

SEX WISE DISTRIBUTION OF HER-2/ neu EXPRESSION



It is seen from the above clustered column diagram that HER-2/neu positivity was significantly higher in females (100%) than in males in which HER-2/neu positive was 52% and negative was 48%. These observations were statistically significant.($p<0.05$)

TABLE 9

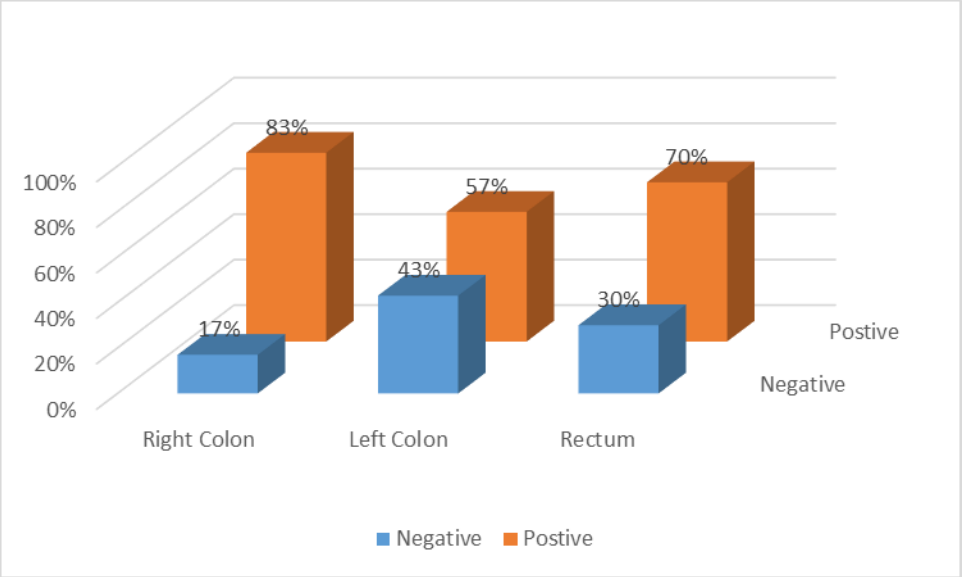
HER-2/ neu EXPRESSION IN VARIOUS LOCATIONS

HER-2/neu					
SITE	**-VE**	**+1**	**+2**	**+3**	**+VE(%)**
Right Colon	1 (17%)	1	1	3	83%
Left Colon	6 (43%)	5	1	2	57%
Rectum	3 (30%)	1	1	5	70%
Total	10	7	3	10	

From the above table HER-2/neu positivity was higher in right colon with 5 cases (83%) out of 6 cases, followed by rectum with HER-2/neu positivity in 7 cases (70%) out of 10 cases and left colon with HER-2/neu positivity in 8 cases (57%) out of 14 cases. Chi square analysis showed a stastically insignificant result. ($p>0.05$)

CHART 9

HER-2/ neu EXPRESSION IN VARIOUS LOCATIONS



As depicted in above clustered cylinder column, HER-2/neu immunoreactivity was expressed in right colon by 83% followed by rectum by 70% and left colon by 57%.Chi square analysis showed a statistically insignificant result.($p>0.05$)

TABLE 10

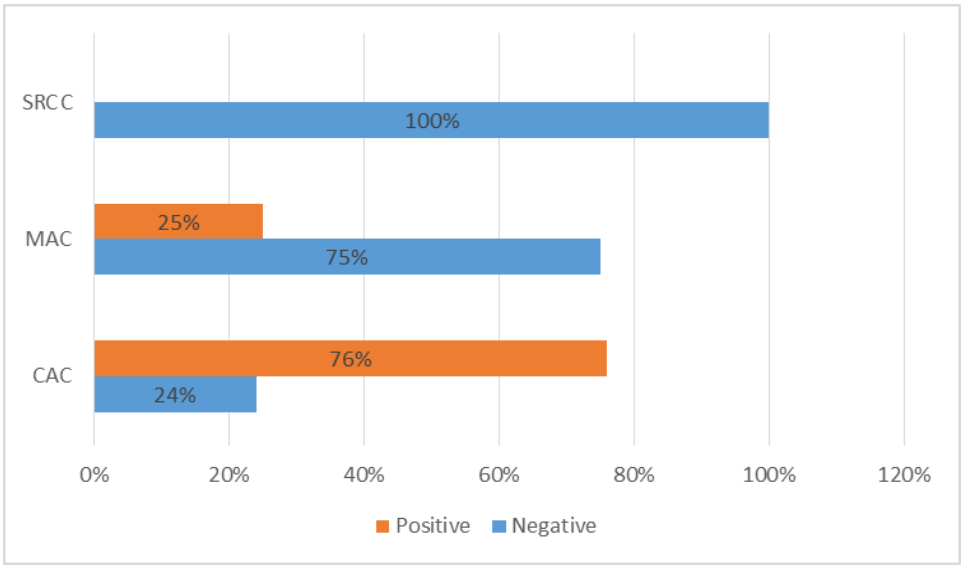
HER-2/ neu EXPRESSION IN VARIOUS TYPES

HER2/neu					
TYPES	**-VE**	**+1**	**+2**	**+3**	**+VE(%)**
CAC	6 (24%)	7	3	9	76%
MAC	3 (75%)	0	0	1	25%
SRCC	1 (100%)	0	0	0	0%
Total	10	7	3	10	

As seen in the above table, HER-2/neu positivity was 76% in conventional adenocarcinoma followed by mucinous adenocarcinoma showing 25% HER-2/neu positivity. Only one case of signet ring cell carcinoma was observed which was HER-2/neu negative.These results were statistically insignificant.($p>0.05$)

CHART 10

HER-2/ neu EXPRESSION IN VARIOUS TYPES



In the present study as seen from the bar chart, conventional adenocarcinoma showed 76% HER-2/neu positivity, mucinous adenocarcinoma showed 25% HER-2/neu positivity. Signet ring cell carcinoma was seen in only one case which is HER-2/neu negative. Chisquare analysis was statistically insignificant.($p>0.05$)

TABLE 11

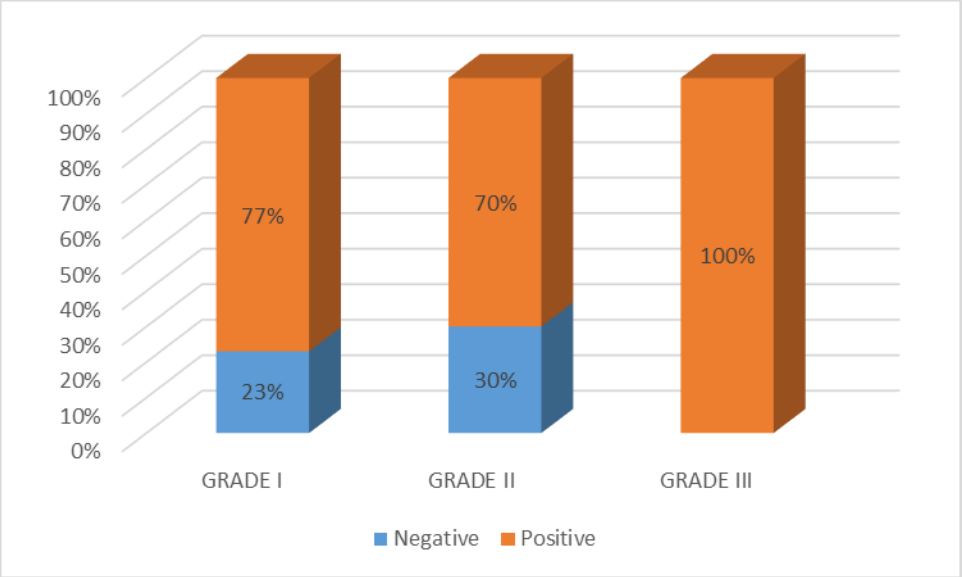
HER-2/ neu EXPRESSION IN VARIOUS GRADES

HER2/neu					
GRADE	-VE	+1	+2	+3	+VE(%)
I	3 (23%)	4	1	5	77%
II	3 (30%)	3	1	3	70%
III	0	0	1	1	100%
Total	6	7	3	9	

It is observed from the above frequency table,out of 13 grade I tumours HER-2/neu positivity was observed in 10 cases(77%). In grade II tumours out of 10 cases, 7 cases (70%) showed HER-2/neu positivity.2 cases of grade III tumours were observed of which both (100%) showed HER-2/neu positivity. These observations were statistically significant. ($p<0.05$)

CHART 11

HER-2/ neu EXPRESSION IN VARIOUS GRADES



As seen from the bar chart, HER-2/neu positivity was 77% in grade I tumours, 70% in gradeII tumours and 100% in gradeIII tumours. Chi square analysis found to be statistically significant.($p<0.05$)

TABLE 12

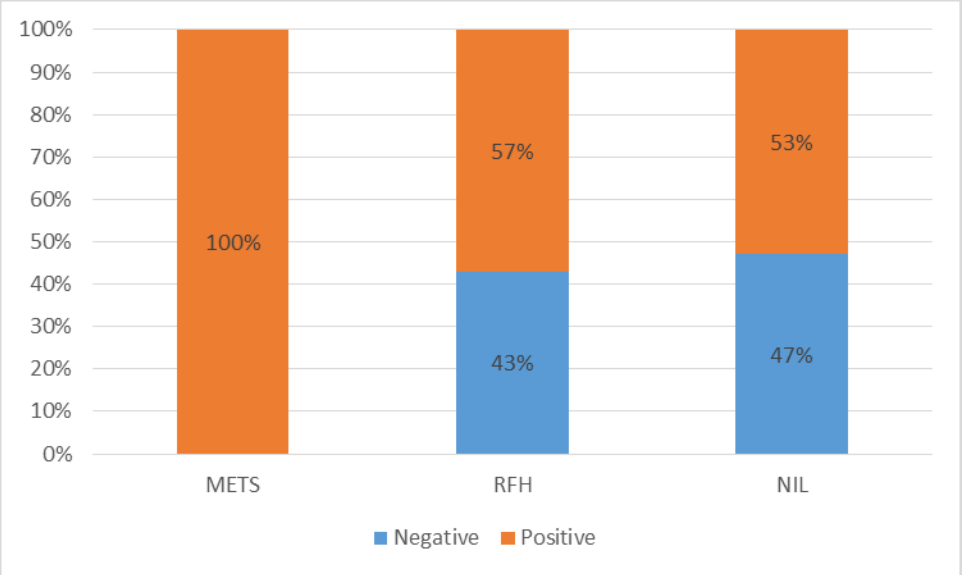
HER-2/ neu EXPRESSION IN LYMPHNODE METASTASES

HER2/neu					
LYMPHNODE	**-VE**	**+1**	**+2**	**+3**	**+VE(%)**
Metastases	0	2	3	3	100%
Reactive	3 (43%)	3	0	1	57%
NIL	7 (47%)	2	0	6	53%
Total	10	7	3	10	

In the present study lymphnode metastases was observed in 8 cases in which all 8 cases(100%) showed HER-2/neu postivity. Out of 7 cases with reactive nodes 4 cases(57%) were HER-2/neu positive.In 15 cases no nodes were recovered. These observations were statistically significant.($p<0.05$)

CHART 12

HER-2/ neu EXPRESSION IN LYMPHNODE METASTASES



It is observed from the above clustered column diagram HER-2/neu postivity was seen in all the 8 cases(100%) which showed lymphnode metastases. In the 7 cases with reactive nodes, 4 cases(57%) showed HER-2/neu postivity.Chi square analysis was statistically significant($p<0.05$)

TABLE 13

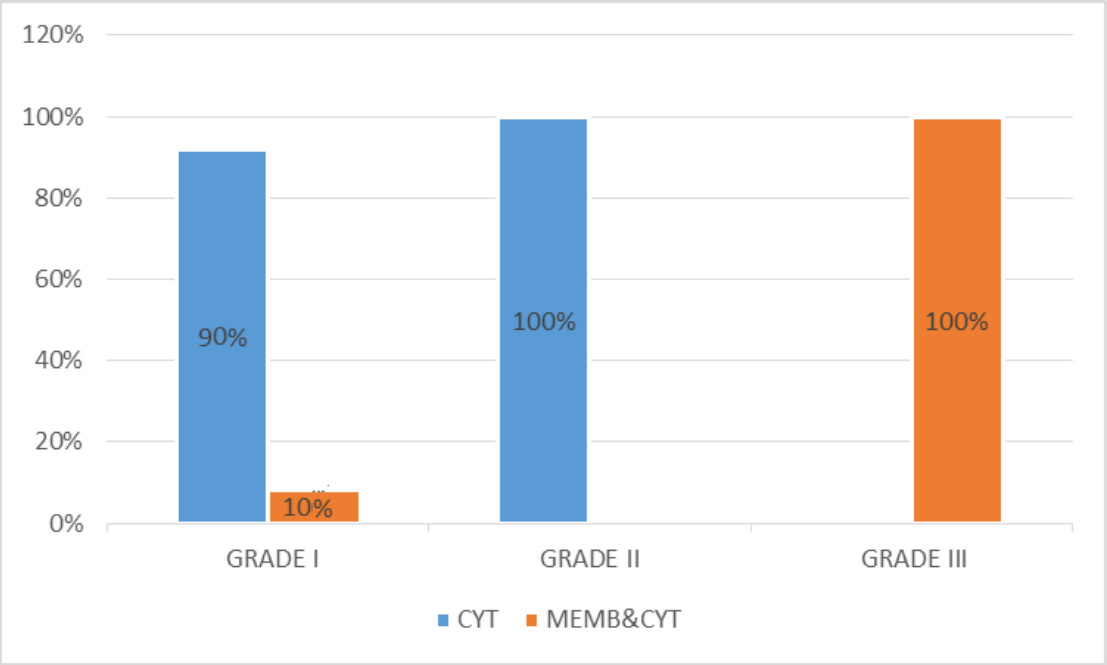
HER-2/ neu PATTERN OF STAINING

PATTERN	GRADE		
	I	II	III
CYT	9 (90%)	7 (100%)	0
MEMB&CYT	1 (10%)	0	2 (100%)
Total	10	7	2

It is observed from the above table, cytoplasmic positivity was seen in 9 cases (90%) of grade I tumours and 7 cases (100%) of grade II tumours. Membranous and cytoplasmic positivity was seen in 1 case (10%) of grade I tumour and 2 cases (100%) of grade III tumours. These results were statistically significant. ($p<0.002$)

CHART 13

HER-2/ neu PATTERN OF STAINING



In the present study cytoplasmic postivity was seen in 90% of grade I tumours and 100% of grade II tumours.Membranous and cytoplasmic positivity was observed in 10% of grade I tumours and 100% of grade III tumours. Chi square analysis was found to be statistically significant.

COLOUR PLATES

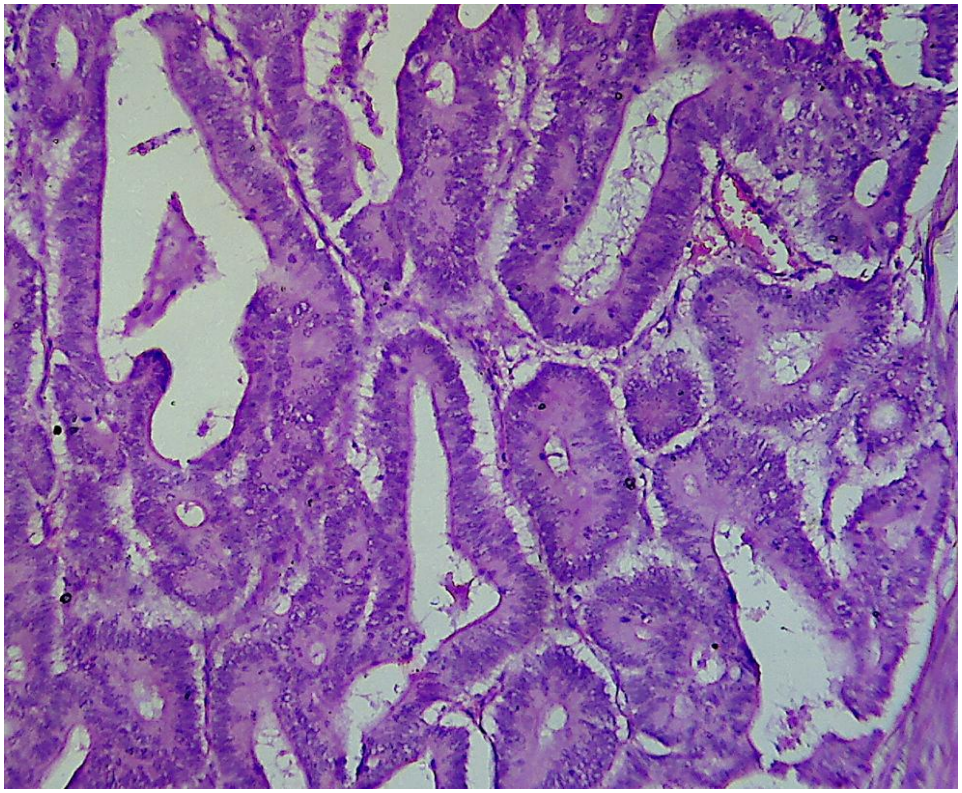


Fig.1. Well differentiated adenocarcinoma of colon H & E(10X)

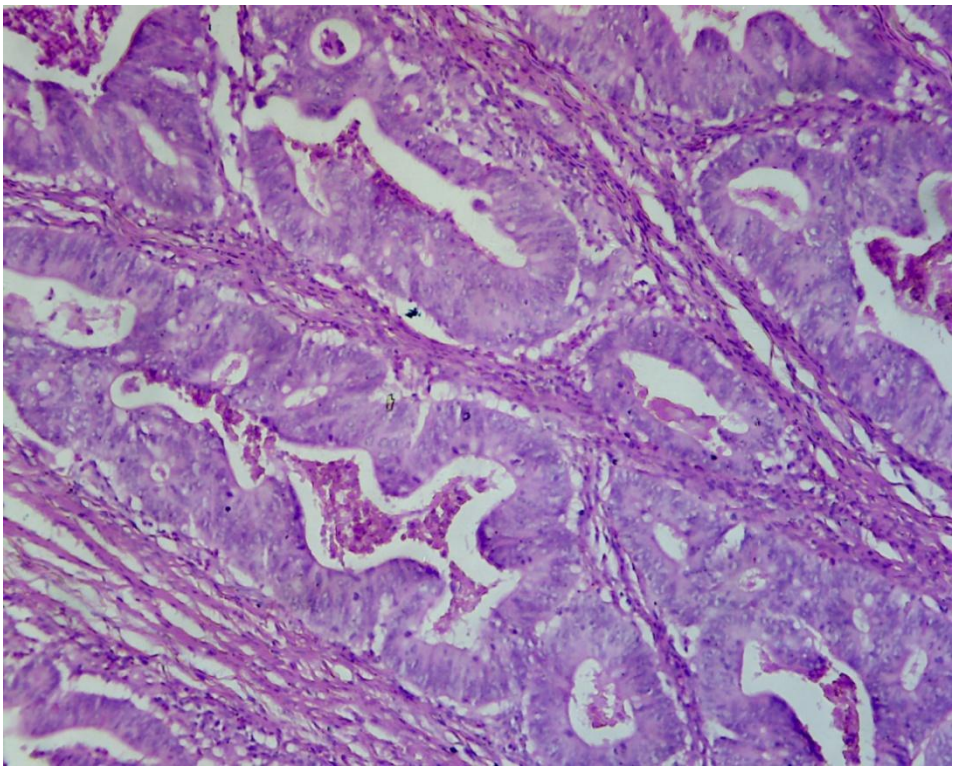


Fig. 2. Moderately differentiated adenocarcinoma of colon H & E(10X)

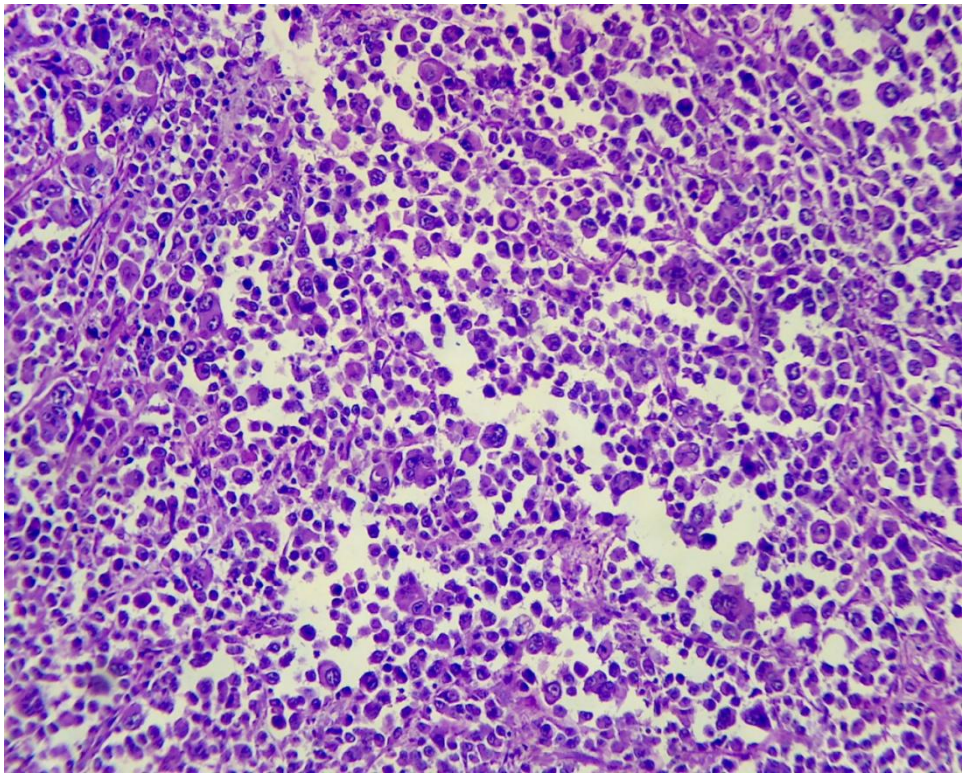


Fig.3. Poorly differentiated adenocarcinoma of colon H & E(10X)

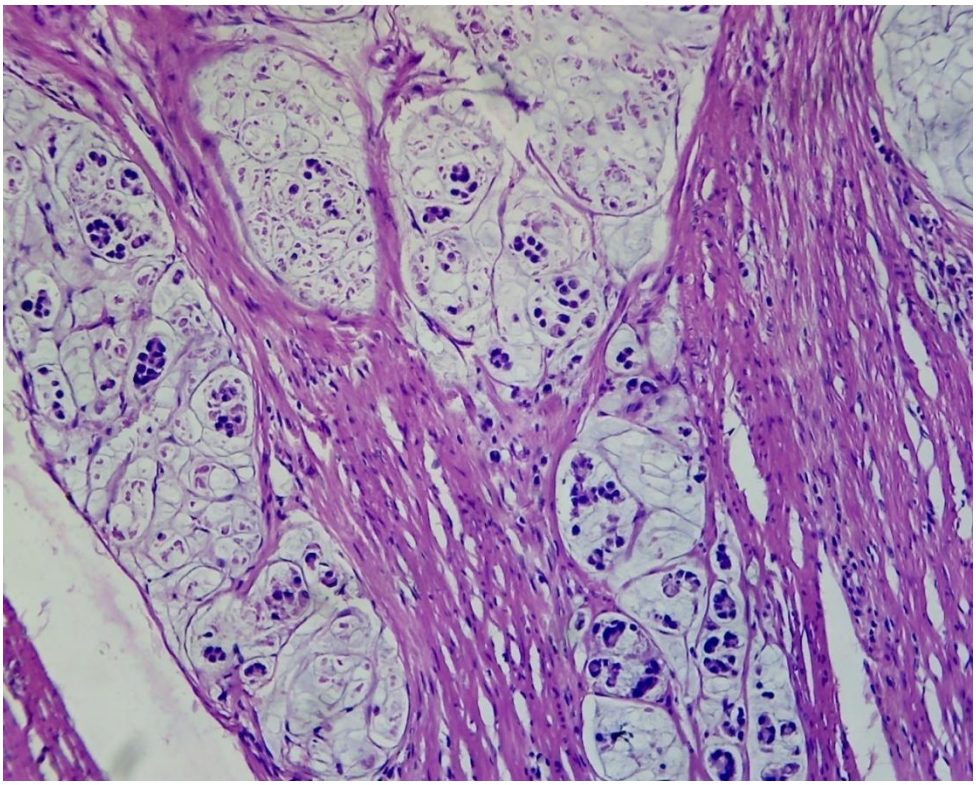


Fig.4. Mucinous adenocarcinoma of colon H & E(10X)

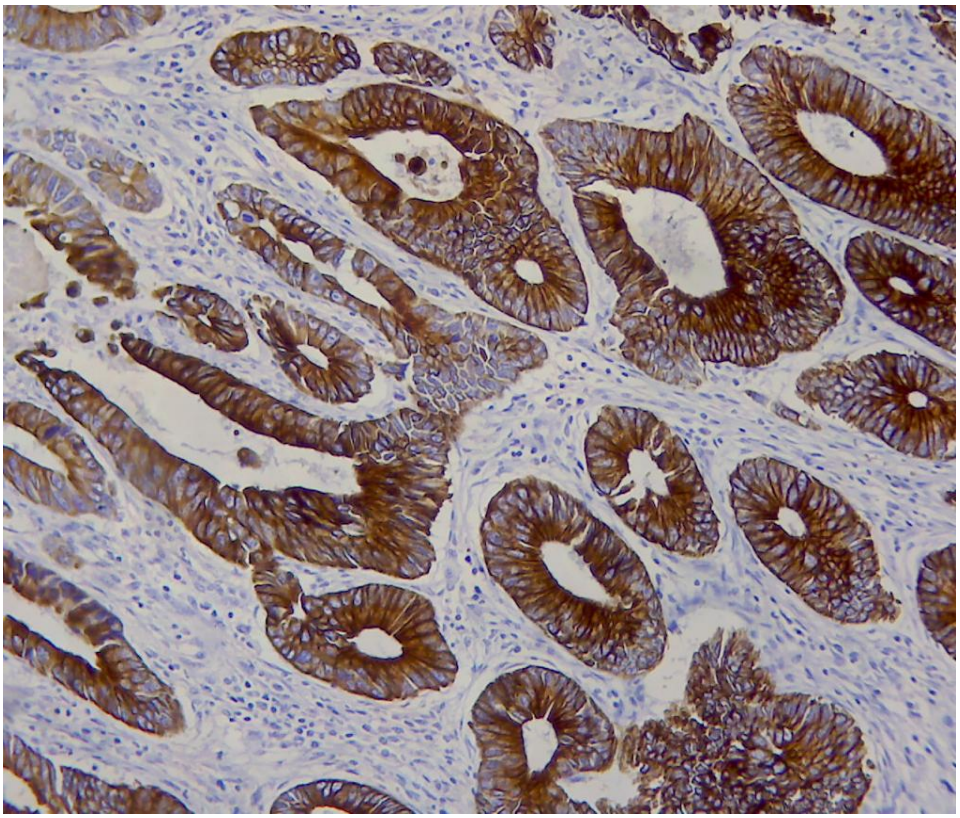


Fig.5. Diffuse (+3)HER-2/neu positivity in Grade I adenocarcinoma showing membranous and cytoplasmic staining(10X)

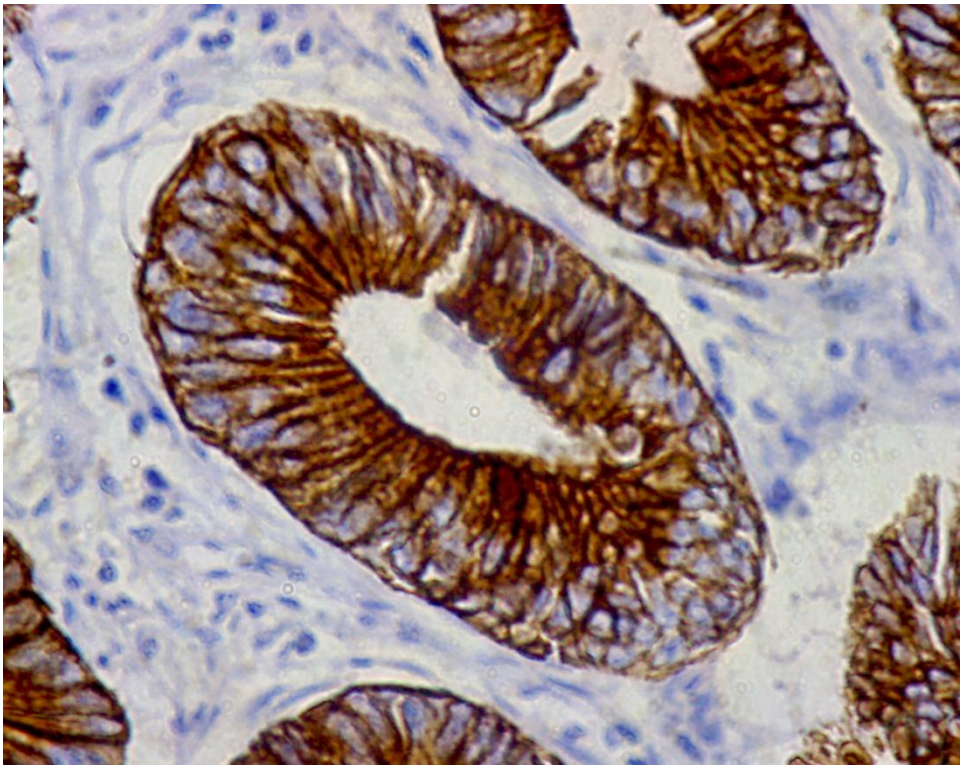


Fig.6. Diffuse (+3)HER-2/neu positivity in Grade I adenocarcinoma showing membranous and cytoplasmic staining (40X)

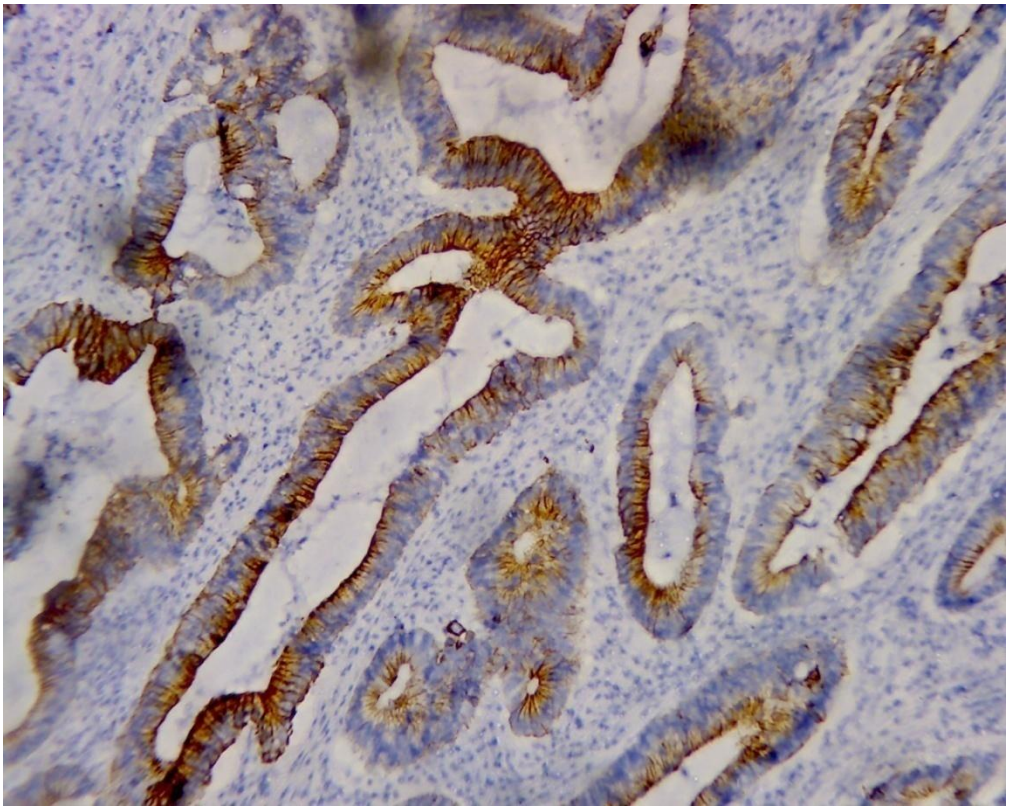


Fig.7. Diffuse (+3)HER-2/neu positivity in Grade II adenocarcinoma showing cytoplasmic staining (10X)

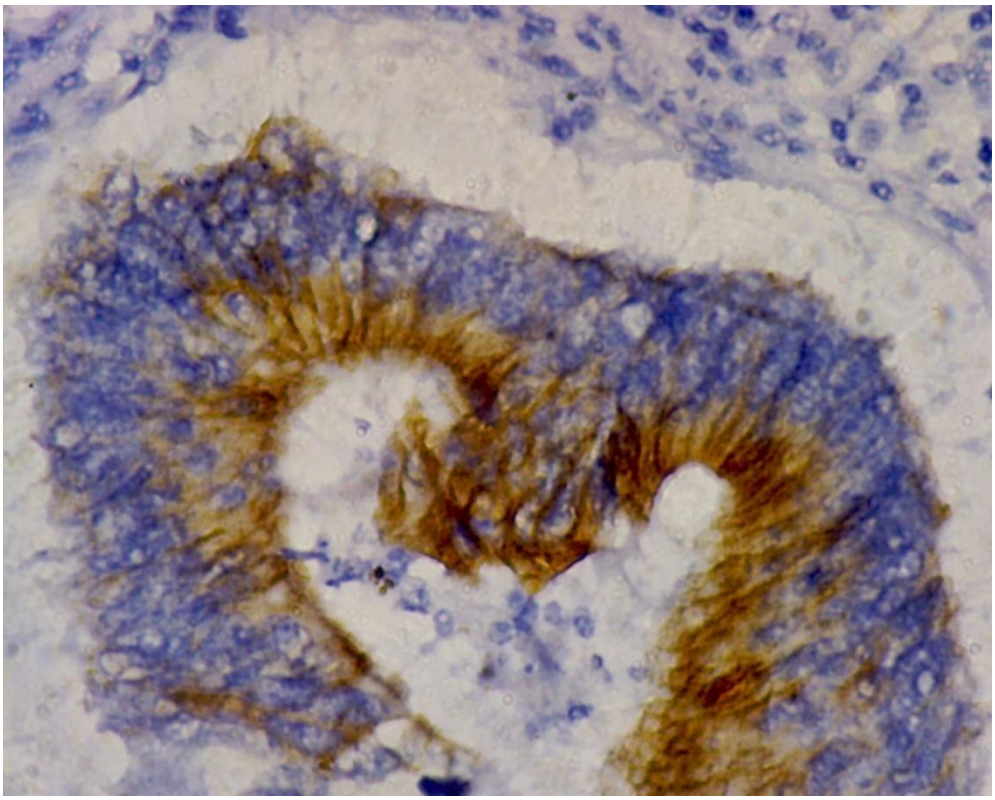


Fig.8. Diffuse (+3)HER-2/neu positivity in Grade II adenocarcinoma showing cytoplasmic staining (40X)

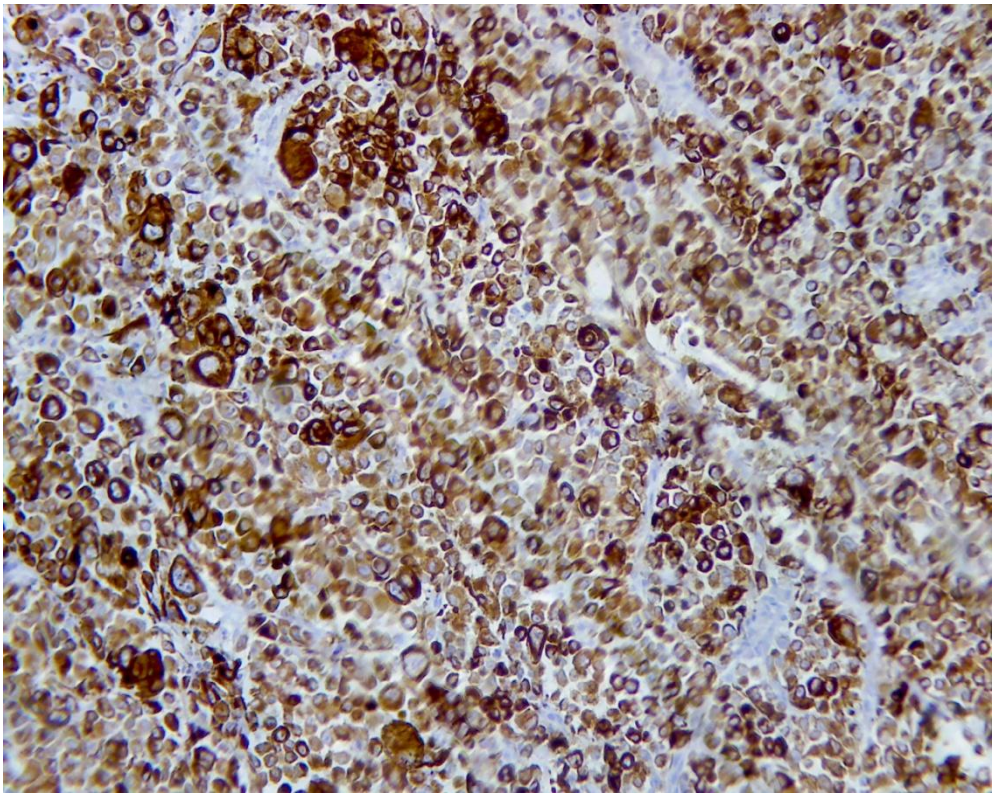


Fig.9. Diffuse (+3)HER-2/neu positivity in Grade III adenocarcinoma showing membranous and cytoplasmic staining (10X)

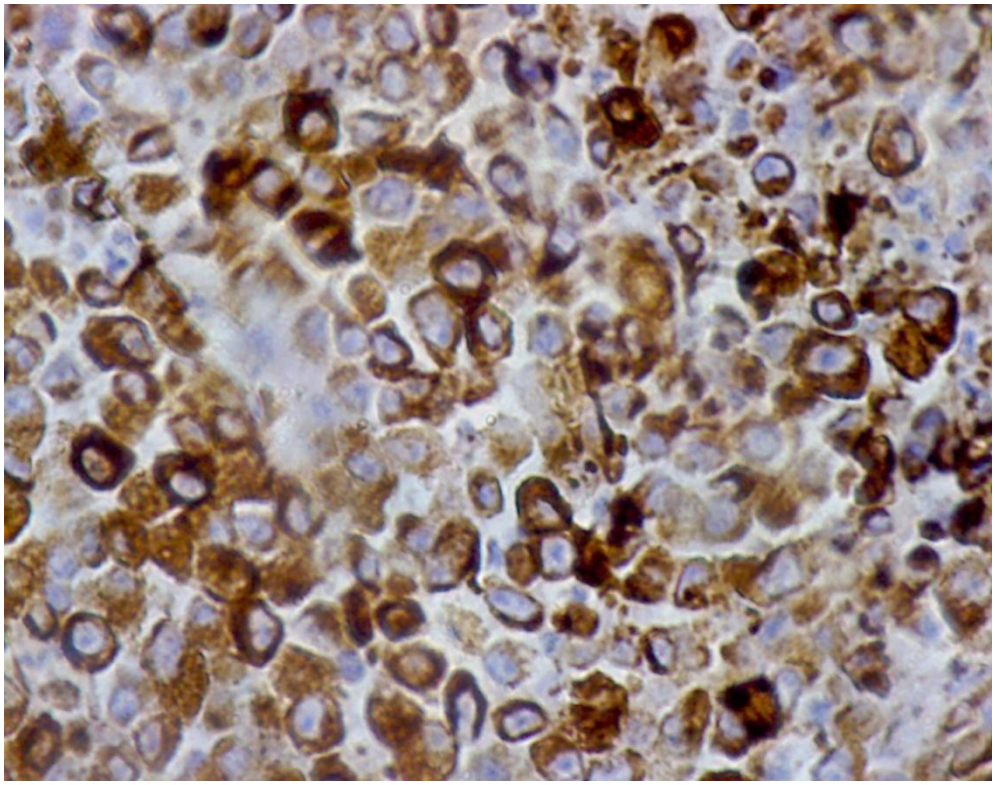


Fig.10. Diffuse (+3)HER-2/neu positivity in Grade III adenocarcinoma showing membranous and cytoplasmic staining(40X)

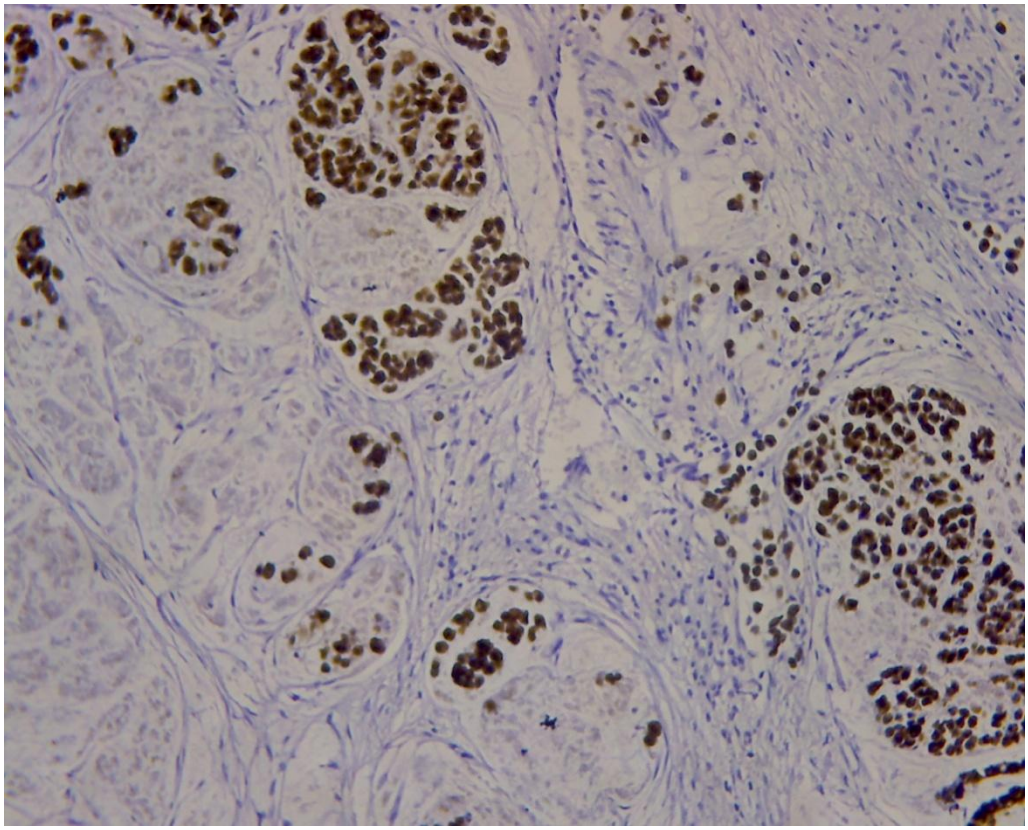


Fig.11. HER-2/neu positivity in mucinous adenocarcinoma showing cytoplasmic staining (10X)

DISCUSSION

DISCUSSION

Colorectal cancer is one of the most common leading causes of cancer related mortality and account for approximately 9% of all human cancers. Eventhough the tumour is diagnosed by histopathology and tumour grade and stage are strong prognostic factors, identification of newer prognostic indicators such as expression of various immunological markers arises depending on which clinical behaviour and treatment differs. Various literature have shown that detection of HER-2/neu is a useful marker and is used to predict the outcome of colorectal cancers.

In the present study, expression of HER-2/neu was studied in colorectal carcinomas and its correlation with other clinicopathological variables were analysed.

Thirty cases of colorectal carcinomas were included in the study. The age of patients ranges from 34 to 80 years with mean age 58.6+/-24.6 years (TABLE 1). This is in accordance with the results by Manmeet Kaur Gill et al (2011) in which mean age was 53.9 years with a range of 19 to 88 years.[3] Similar studies conducted by Ghaffarzadegan et al (2006) and Neklason et al (2008) showed mean age of 56.8 and 55.7 years respectively .[7][84]

Out of 30 cases studied, males were 21 outnumbering females who were 9 in number (ie) males constituted around 70% and females were 30%. The male:female ratio is 2.3:1 (TABLE 2). This is similar to the study by Dalal a.elwy et al (2012) in which out of 50 cases 60% were males and 40% were females with a male to female ratio 1.5:1.[8] Study by Schuell et al (2006) also showed a male preponderance.[85]

Out of 30 cases studied, involvement of right colon is seen in 20% and 80% in left colorectal region (Table 3). Tavangar and associates (2005) also observed similar results with left colorectal region involvement constituting 54.4% and right colon constituting 45.6%.[86]

The study includes following types of adenocarcinomas, conventional, mucinous and signet ring cell carcinoma. Of these conventional adenocarcinoma was most common constituting about 83.3% followed by mucinous adenocarcinoma constituting 13.40% and signet ring cell carcinoma 3.3% (TABLE 4). A Study by Manmeet Kaur Gill et al (2011) showed similar results as in the present study with conventional adenocarcinoma constituting 77.5% followed by mucinous adenocarcinoma 17.5% and 2.5% of each signet ring cell carcinoma and carcinoid.[3]

Out of 30 cases, 25 cases were conventional adenocarcinoma of which 52% was grade I tumour, 40% was grade II tumour and 8% was grade III tumours.(Table 5). Similar results were observed in studies by Manmeet kaur Gill et al and and Ghaffarzadegan et al.

Out of total thirty cases studied 20 cases were HER-2/neu positive and 10 cases were HER-2/neu negative which constitutes 67% and 33% respectively (TABLE 6). Out of 67% positive cases 31% showed +3 staining, 13% showed +2 staining and 23% showed +1 staining for HER-2/neu. The staining intensity in most cases was moderate (45%) to strong (19.5%) with weak staining in only one case (2.5%). 56.9% of cases showed cytoplasmic positivity and 10.1% showed membranous and cytoplasmic staining. No case showed pure membranous pattern of staining.

Similar results were observed in a study by Manmeet Kaur Gill et al (2011) in which HER-2/neu positivity constituted about 65% of which cytoplasmic staining was seen in 57.5% and membranous-cytoplasmic staining in 7.5%. As in our study, no case showed a pure membranous pattern of staining. 30% of cases showed +3 staining, 12.5% showed +2 staining and 22.5% showed +1 staining. Intensity of staining was moderate (50%) to strong (12.5%) with weak staining in one case

(2.5%).[3] In another study by Ghaffarzadegan et al (2006) in 69 cases, HER-2/neu positivity was seen in 59.4% with cytoplasmic staining, membranous and cytoplasmic staining in 34.1% of cases.[84] Another study by Half et al (2003) cytoplasmic staining seen in 65.9% and membranous staining in 5% of cases.[87] Our study correlates well with all the above studies.

In contrast to our study Nathanson et al (2003) studied HER- 2/neu expression and gene amplification in 139 cases and found only five cases (3.6%) showed HER-2/neu positivity. The reason for this low positivity might be they have considered only membranous staining.[88]

HER-2/neu positivity was analysed with the age of the patients. Out of 30 cases, 7 cases were in age group of 31-50 years of which 4 (57%) were HER-2/neu positive, 20 cases in age group of 51-70 years of which 13(65%) were HER-2/neu positive and 3 cases in age group >70 years in which all the 3(100%) were HER-2/neu positive. It was seen that as the age of the patients increased, the percentage positivity of HER-2/neu staining also increased. These results were analysed by chi-square test analysis and was found to be statistically significant ($P<0.03$) (TABLE 7). Similar results were seen in study by Manmeet Kaur Gill et

al (2011) in which HER-2/neu percentage positivity was increased as age increased with a p value of 0.002.[3]

HER-2/neu positivity was analysed with sex of the patients. Of the total 30 cases, 21 were males out of which HER-2/neu positivity is seen in 11 cases which constituted around 52%. 9 cases were females of which all of them (100%) showed HER-2/neu positivity. (TABLE 8). In a study by Gruenberger et al (2006) percentage positivity was more in males than in females which was in contrast to our study.[89]

In the present study, out of 30 cases involvement of right colon was seen in 6 cases of which 5 cases (83%) showed HER-2/neu positivity. Left colon involvement was seen in 14 cases of which 8 cases (57%) showed HER-2/neu positive. Rectum was involved in 10 cases out of which 7 cases (70%) showed HER-2/neu positivity. Chi-square analysis showed a statistically insignificant relation between HER-2/neu expression and site of tumour.($p>0.05$). (TABLE 9). These results were similar to other study by Mohammadi et al (2011) in which no statistically significant correlation was obtained between HER-2/neu expression and site of tumour.[90]

Types of colorectal adenocarcinoma observed in the present study were conventional adenocarcinoma which showed HER-2/neu positivity

by 76% followed by mucinous adenocarcinoma 25%. Only 1 case of signet ring cell carcinoma was observed which was HER-2/neu negative. Chi-square test showed a p value >0.05 which was statistically insignificant (TABLE 10). Similar results were observed in a study by Kavanagh et al (2009) in which there was no statistically significant relation was seen between HER-2/neu expression and histologic types.[91]

In the present study, Grade I tumours showed 77% of HER-2 neu positivity followed by grade II tumours constituting about 70% and grade III tumours showing 100% positivity for HER-2/neu.(TABLE 11). These results were similar to studies by Manmeet Kaur Gill et al (2011) and Hay et al (2003) in which they found significant correlation between HER-2/neu and grade. They also observed that grade III tumours showed 100% HER-2/neu positivity.[3]

In present study, membranous and cytoplasmic positivity was seen 100% in grade III tumours than in grade I tumours which constitutes 10%. A study by Shoroq Mohammed Abas al (2014) also showed 100% membranous and cytoplasmic HER-2/neu positivity in grade III tumours more than grade I.[92] Similar results were also observed by Ghaffarzadegan et al (2006).[84] In the present study, out of 30 cases, only in 15 cases nodes were recovered and 8 out of them were metastatic.

All the 8 cases with metastatic nodes were positive for HER-2/neu staining. Out of 7 reactive nodes only 4 cases was HER-2/neu positive. These results were statistically significant ($p<0.05$) and that there is correlation between HER-2/neu staining and lymph node metastases (TABLE 12). This was similar to study by Manmeet Kaur Gill et al (2011) in which out of 40 cases in 20 cases lymphnodes were recovered and ten were metastatic and all the ten cases were HER-2-neu positive.[3]

SUMMARY & CONCLUSION

SUMMARY AND CONCLUSION

Colorectal carcinomas are one among the most common human malignancies. Most of the tumours are diagnosed, classified and graded with H & E stained sections. Various immunological markers are expressed in colorectal malignancies and these are studied to identify targeted therapy and to increase the survival of the patients. HER-2/neu overexpression indicates higher tumour grade and stage and a monoclonal antibody (Trastuzumab) directed against HER-2 has increased survival in many tumours. Hence HER-2/neu expression in colorectal carcinomas are being studied extensively.

In the present study thirty cases of colorectal adenocacinomas were included. HER-2/neu positivity is seen in 67% of the cases. HER-2/neu expression increased as age of the patients increased. HER-2/neu expression was higher in females (100%) compared to males. Location and type of tumour had no correlation with HER-2/neu. HER-2/neu staining was higher in grade III tumours(100%) when compared to 77% in grade I tumours showing a positive correlation of HER-2/neu with grade. This suggests as the tumour grade increases HER-2/neu positivity increases. There was 100% HER-2/neu positivity in metastatic nodes

showing a positive correlation. Membranous and cytoplasmic staining was higher (100%) in grade III tumours.

From the study it is concluded that rate of HER-2/neu expression in colon carcinoma is high. Most cases had cytoplasmic staining but membranous and cytoplasmic staining was seen in higher grades. Hence targeted therapy could be helpful in patients with high grade and with lymph node or distant metastases. Because of many pitfalls in immunohistochemistry, further studies such as gene amplification studies involving larger number of patients are needed to assess HER-2/neu expression in colorectal carcinomas and to develop new targeted therapy.

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ANNEXURE I

ANNEXURE I

PROFORMA

COIMBATORE MEDICAL COLLEGE

DEPARTMENT OF PATHOLOGY

COIMBATORE

Particulars of the patient:

Name : IP/OP number :

Age(Years) : Ward No :

Sex : Occupation :

Address :

Presenting Complaints and Duration:

Abdominal pain

Change in bowel habits with alternate diarrhea and constipation

Hematochezia

Easy fatiguability

Fever

Loss of weight/Loss of appetite

Past history:

History of previous surgeries

History of previous irradiation

Family history of any malignancy

Personal history:

Dietary habits

Smoker +/-

Alcoholic +/-

General Physical Examination:

Built	:	Febrile/afebrile	:
Nourishment	:	Pallor	:
Conscious	:	Jaundice	:
Weight	:	Cyanosis	:
Pulse Rate	:	Clubbing	:
Respiratory rate	:	Lymphadenopathy	:

Systemic Examination :

RS : P/A :

CVS : CNS :

Digital Rectal Examination:

Radiological Findings:

X-Ray :

CT :

MRI :

Colonoscopic Findings :

Microscopic Findings:

Histopathological Diagnosis :

Immunohistochemistry :

FINAL DIAGNOSIS

CONSENT FORM

Dr.N.Vani,postgraduate student in the department of pathology,Coimbatore Medical College is conducting a study on "**HER 2 neu EXPRESSION IN COLORECTAL ADENOCARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGIC VARIABLES".** Colectomy done for various colorectal lesions are received in pathology department and are processed and examined under a microscope to obtain diagnostic information or is tested for other studies. I have been informed to my satisfaction regarding the nature of procedure. The data used herein may be used for research and publication.

Name :

Place :

Signature :

ஒப்புதல் படிவம்

பெயர் .

வயது .

பாலினம் .

முகவரி .

அரசு கோவை மருத்துவக் கல்லூரியில் நோய் குறியியல் மருத்தவ துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவி **மரு.ந.வாணி** அவர்கள் மேற்கொள்ளும் **"பெருங்குடல் புற்றுநோயில் Her-2/neu வின் வெளிப்பாட்டினை அறிதல்"** பற்றிய ஆய்வில் செய்முறை மற்றும் அனைத்து விளக்கங்களையும் கேட்டுக் கொண்டு எனது சந்தேகங்களை தெளிவுப்படுத்திக் கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன்.

நான் இந்த ஆய்வில் முழு சம்மதத்துடனும், சுய சிந்தனையுடனும் கலந்து கொள்ள சம்மதிக்கிறேன்.

இந்த அய்வில் என்னைப் பற்றிய அனைத்து விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதை தெரிவித்துக் கொள்கிறேன். எந்த நேரத்திலும் இந்த ஆய்வில் இருந்து நான் விலகிக் கொள்ள எனக்கு உரிமை உண்டு என்பதையும் அறிவேன்.

இடம்

தேதி கையொப்பம் / ரேகை

ANNEXURE II

ANNEXURE - II

MASTER CHART

S.NO	AGE	SEX	IPNO	HPENO	SITE	TYPES	GRADE	HER2NEU	LN METS
1	47	M	45521	304/14	LC	CAC	II	1+	METS
2	67	M	3482	305/14	REC	CAC	I	1+	RFH
3	65	M	14203	950/14	LC	CAC	II	0	-
4	54	M	10650	1053/14	REC	CAC	I	3+	METS
5	34	M	11614	1209/14	REC	CAC	I	0	-
6	53	F	23095	1470/14	REC	CAC	II	3+	-
7	78	M	27178	2206/14	LC	CAC	I	1+	-
8	60	M	46997	2760/14	RC	CAC	III	2+	METS
9	58	M	51336	3079/14	LC	CAC	II	0	-
10	54	M	51403	3215/14	LC	CAC	II	1+	RFH
11	65	M	56708	3227/14	LC	CAC	III	3+	METS
12	70	M	62254	3530/14	LC	CAC	II	0	RFH
13	67	F	76055	4210/14	RC	CAC	II	3+	-
14	47	M	76031	4211/14	LC	CAC	I	0	-
15	58	F	81839	262/15	REC	CAC	I	3+	-

16	62	F	1684	280/15	RC	CAC	II	1+	-
17	40	M	5143	324/15	LC	MAC	-	0	RFH
18	50	F	81981	330/15	LC	CAC	I	1+	METS
19	60	M	5785	405/15	RC	MAC	-	0	-
20	60	F	10825	702/15	REC	CAC	I	3+	-
21	65	M	7654	705/15	LC	CAC	II	2+	METS
22	42	F	16141	859/15	RC	CAC	II	3+	RFH
23	40	F	22848	1194/15	RC	MAC	-	3+	-
24	70	M	32821	1805/15	REC	CAC	I	0	RFH
25	60	M	36174	1911/15	LC	MAC	-	0	-
26	64	M	38268	2136/15	REC	CAC	I	2+	METS
27	72	M	41223	2187/15	LC	CAC	I	3+	METS
28	55	M	43589	2274/15	REC	SRCC	-	0	-
29	62	F	46634	2460/15	LC	CAC	I	1+	RFH
30	80	M	51147	2499/15	REC	CAC	I	3+	-

KEY WORDS TO MASTER CHART

LC - Left colon

RC - Right colon

REC - Rectum

CAC - Conventional adenocarcinoma

MAC - Mucinous adenocarcinoma

SRCC - Signet ring cell carcinoma

METS - Metastases

RFH - Reactive follicular hyperplasia

ANNEXURE III

ANNEXURE III-LIST OF ABBREVATIONS

H & E - Hematoxylin and Eosin

HER-2/neu - Human Epidermal growth factor Receptor

CK - Cytokeratin

CEA - Carcino Embryonic Antigen

HCG - Human Chorionic Gonadotrophin

PLAP - Placental Alkaline Phosphatase

APC - Adenomatous Polyposis Coli

TAG 72 - Tumour Associated Glycoprotein

ACF - Aberrant Crypt Foci

CAC - Conventional Adenocarcinoma

MAC - Mucinous Adenocarcinoma

SRCC - Signet Ring Cell Carcinoma

METS - Metastases

RFH - Reactive Follicular Hyperplasia

CYT - Cytoplasmic

MEMB - Membranous